Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones

National Cemetery Administration Final Report October 24, 2011 Mary F. Striegel, Chief, Materials Research, NCPTT and Jason W. Church, Materials Conservator, Materials Research, NCPTT



Jefferson Barracks National Cemetery, St. Louis, Mo.

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Section 1

Comparative Evaluation of Commercially Available Cleaners for Use on Government Issued Headstones

EXECUTIVE SUMMARY

In 2004, The Department of Veterans Affairs (VA), National Cemetery Administration (NCA) was facing rising costs associated with maintenance of national cemeteries. NCA staff was looking for more effective ways to maintain headstones to meet VA National Shrine Standards. Historic and older stones were showing signs of accelerated weathering. Headstones were being replaced because of surface deterioration and loss, as well as lack of readability, and other conditions. Manpower costs were increasing and work was being shifted from VA cemetery maintenance crews to contractors. Additionally, a range of cleaning products was being used from region to region and cemetery to cemetery.

Based on these considerations, NCA was interested in developing a research program that would help them make informed cleaning decisions. By evaluating cleaning methods and drawing from information developed by others, they hoped to (1) identify the most appropriate and effective long-term treatments that would minimize the frequency of cleaning cycles; and (2) increase the lifetime of headstones by minimizing damage due to regular cleaning and maintenance.

The National Park Service's National Center for Preservation Technology and Training (NCPTT) began a national cemetery preservation initiative in 2003 in efforts to improve technologies for the preservation of public and private cemeteries. NCPTT had in-house research capabilities and access to leading preservation professionals who worked in historic cemeteries. As part of the initiative, NCPTT launched a series of cemetery monument conservation workshops and seminars.

NCPTT and NCA developed and implemented a research plan to evaluate five commercially available cleaners based on products currently used in both national and historic cemeteries. Research was implemented in three somewhat overlapping phases. NCA maintenance managers were surveyed to determine products that were being used in the field and preservation professionals offered suggestions for cleaning products. From a list of more than 30 products, NCPTT and NCA staff chose five products to evaluate based on common use in the field, chemical cleaning mechanism, pH, and other characteristics. NCPTT and NCA chose to study D/2 biological solution, Daybreak cleaner, World Environmental Group's Marble and Granite Cleaner, H2Orange Grout Safe Cleaner, and Kodak Photo-flo.

In Phase One, products were evaluated based on (1) cost effectiveness, (2) environmental safety, (3) ability to clean stones and inhibit biological regrowth, (4) ease of use, and (5) potential to lower stone damage. Colorado Yule and Georgia Cherokee marble were selected for study since they were commonly found in national cemeteries. Tests took into consideration geographic and climatic conditions, as well as localized environments such as sunny or shady areas. Microbiological methods were used to identify microorganisms present before and after cleaning. These techniques were used to follow biological activity and identify regrowth as it occurred in the field. Also, color measurement and

visual appearance techniques were used to evaluate headstones in the field. Most field testing took place in Phase One of the study, and ran from April 2005 to November 2006. Results of this work are found in the *Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones, Progress Update Report*, dated March 2007. At the end of Phase One, two cleaners were eliminated: H2Orange Grout Safe Cleaner, and Kodak Photo-flo.

In Phase Two of the study, NCPTT developed laboratory tests to evaluate physical and chemical changes to both field and laboratory marble samples. A wide variety of analytical methods were implemented. Laboratory testing included microscopy, conductivity, colorimetry, profilometry, porosimetry, and artificial weathering tests. Phase Two results are presented in the report, *Phase II: Chemical and Physical Testing for the Evaluation of Effects of Cleaners on Marble*, October 2011.

Phase Three of the research focused on the long-term regrowth of microorganisms on stone surfaces after cleaning. This phase was originally intended to take place in the field in Jefferson Barracks National Cemetery and in Santa Fe, National Cemetery. Long term monitoring was planned. Unfortunately, through a series of unforeseen events, headstones to be included in this phase were cleaned at four of the five national cemeteries included in the study. An alternate approach was devised. In September 2009, the School of Engineering and Applied Sciences, Harvard University, was contracted to undertake an accelerated laboratory study to evaluate the antimicrobial activity of three cleaners on marble. The final results of this phase are found in *A Report on an Evaluation of Antimicrobial Activity of Three Biocides on Marble*, December 2010.

Results

A summary of the evaluation and performance of cleaners can be found in the attached Table. The summary is derived from data generated in all three phases of this study.

Sunshine Makers D/2 biological solution, a bactericide cleaner based on quaternary ammonium compounds, was the best performer in this comparison of five cleaners. This cleaner was effective at removing biological growth based on field studies in Phase One. While the original formulation of D/2 biological solution did leave fine salts on the surface of stones in the artificial weathering tests of Phase Two research, a reformulated D/2 did not leave residues. In antimicrobial studies of Phase Three, D/2 performed as well as Daybreak and better than WEG Marble and Granite Cleaner after 188 days in a cultured environment.

World Environmental Group, Inc. Marble & Granite Cleaner was environmentally safe and effective in the field. The cleaning action is based on solvents, alcohols, and a chelating agent. It left no salt residues upon artificial weathering. However during antimicrobial studies, fungal regrowth was seen on Colorado Yule marble at 188 days post treatment with the Marble & Granite Cleaner. This product may be a good alternative cleaner to others tested. While Marble & Granite Cleaner is commercially available, the distribution of the product is limited.

Certified Labs Daybreak Cleaner can be harmful to the headstones and to the maintenance crews that use this product. In addition to being a basic cleaner, it contains solvents such as benzene which is a known carcinogen. While it is effective at removing and inhibiting biological growth, as evidenced by field tests and microbiological lab studies, it may cause physical damage to the marble. Soluble salts,

seen in the artificial weathering studies of Phase Two, have the ability to create crystallization pressures in the pores of stone which result in weakening and powdering of stone over time.

H2Orange2 Grout Safe cleaner contains hydrogen peroxide and citrus oil in a cleaning product. It was eliminated from this research in Phase One based on visible biological growth found on headstone test patches at Jefferson Barracks National Cemetery. The test patches displayed green growth six months after initial cleaning.

Kodak Photo-Flo is a surfactant that is commonly used in the photo negative industry. Photo-Flo is a cleaner that acts as a wetting agent and works by decreasing surface tension on the surface of the stone. Use of Photo-Flo for cleaning headstones was recommended by some preservation practitioners. This product was the worst performer in Phase One field studies of the research project and was eliminated from later phases of the work. Headstone patches cleaned with Photo-Flo had the greatest changes in appearance as measured in color measurements. The product was a poor antibacterial agent in both sunny and shady areas. It ranked lowest of all the cleaners in the study.

Recommendations

Using the data from this research, NCPTT has developed best practice recommendations which can be found in the July 2011 report, *Best Practice Recommendations for Cleaning Government Issued Headstones*.

Biocidal cleaners which contain quaternary ammonium compounds, like D/2 Biological Solution manufactured by Sunshine Makers, Enviro Klean® BioWash®, Modec MDF-500 or other cleaners, are preferred products for cleaning marble headstones. This class of cleaners is effective at removing biological growth and general soiling in the field. While all cleaning methods alter the surface to some extent, these cleaners should not harm the stone. It is important to follow the manufacturer's recommendations for dilution ratios and dwell rates. It is important to know that marble cleaned with biocides should continue to lighten over the next few days. The advantage of a biocidal cleaner is that it helps remove a wide range of soiling including biological growth. The disadvantage is that the cleaners are more expensive than other products on the market.

Bleach and bleach-like products, such as Chlorox, Chlorox Outdoor, or Daybreak should not be used to clean marble headstones. While these products are good at killing fungi and other microbes, they are harsh cleaners that leave behind soluble salts. Over time these products will lead to surface loss and powdering of the marble. If cleaning products contain sodium hypochlorite (NaClO), sodium perborate, sodium percarbonate, sodium persulfate, tetrasodium pyrophosphate, calcium hypochlorite or urea peroxide, do not use them for cleaning the headstone.

Some solvent-based products, like World Environmental Group Marble & Granite Cleaner, may be useful at removing general soiling and biological growth from headstones. This is a large class of cleaners, many of which have not been tested on headstones. Marble & Granite Cleaner did not provide long

¹ Exclusively distributed by Cathedral Stone® Products, Inc., 7266 Park Circle Drive, Hanover, MD 21076, Telephone: 410-782-9150, Fax: 410-782-9155.

² Manufactured and distributed by PROSOCO, Inc., 3741 Greenway Circle, Lawrence, KS 66046. Telephone: 800-255-4255; Fax: 785-830-9797. E-mail: CustomerCare@prosoco.com.

term antimicrobial properties. Thus use of this class of cleaners may result in more frequent cleaning of the headstones.

Citrus based products, like H2Orange2 Grout Safe cleaner, were ineffective at preventing regrowth of microorganisms on marble headstones. Citrus oils may even serve as a nutrient source for microorganisms in the long term. Kodak Photo-Flo was also ineffective and not recommended for cleaning headstones.

Section 2

Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones

National Cemetery Administration Progress Update as of March 10, 2007 Mary F. Striegel, Chief, Materials Research, NCPTT and Jason W. Church, Materials Conservator, Materials Research, NCPTT

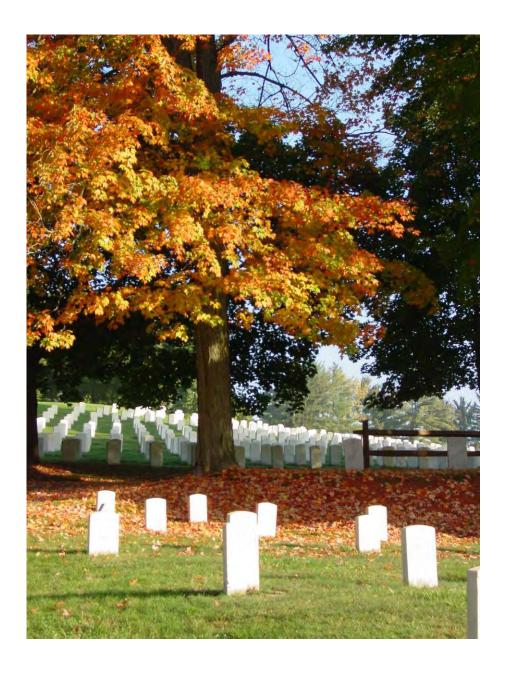


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Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones

National Cemetery Administration Progress Update as of March 10, 2007

This report provides information and progress on the comparative study of commercially available cleaners for federally issued headstones undertaken by the National Center for Preservation Technology and Training and the National Cemetery Administration through March 10, 2007.

1. Executive Summary

In 2004, the Department of Veterans' Affairs, National Cemetery Administration, and the National Center for Preservation Technology and Training entered into an interagency agreement to compare the effectiveness of commercially available cleaners for the removal of soiling and biological growth from Federally-issued headstones. The project goal was to test cleaning products for effectiveness and appropriateness and to make recommendations of products and methods best suited to both clean and preserve the headstones. Main tasks associated with the project were outlined in a project proposal and include both field and laboratory testing over a two-year period.

This study incorporates five national cemeteries that are distributed both geographically and climatically. Cemeteries included in this study are Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM. Cemeteries were chosen to represent various regions of the National Cemetery Administration as well as different climatic zones. The cemeteries include sub-tropical, temperate, continental, semi-arid, and oceanic climates.

Water and five commercially available cleaners, including D/2Antimicrobial cleaner, Daybreak cleaner, World Environmental Group Marble cleaner, H2Orange Grout Safe cleaner, and Kodak Photo-Flo were evaluated at each test cemetery. Cleaners were applied to test patches on headstones carved from Colorado Yule marble and White Cherokee Georgia marble. Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations.

Phase one of the study focused on field trials and ran from April 2005 to November 2006. Changes to headstone test patches as a result of cleaning with test cleaners were evaluated by appearance change and biological activity. Appearance changes were documented using photography and color measurements. Biological activity was documented initially and at six and twelve months after cleaning by enumerating bacteria, fungi and algae taken with BBL culture swabs from a three cm² area from each test patch. The color measurement data was evaluated by calculating the frequency of color changes where ΔE was greater than 5 and where ΔE was greater than 10. These values represent changes that may be perceived by the human eye. Biological activity was presumed to reflect the re-growth of micro-organisms six months after cleaning

(June 2006). The performance of test cleaners was evaluated based on biological activity and was ranked from one to six, with lower numbers indicating poorer performance. Biological activity was again evaluated twelve months after cleaning (February 2007) and the performance of four cleaners were evaluated and ranked.

Based on appearance change data and biological activity data, Kodak Photo-Flo was eliminated from further testing after six months. The greatest number of appearance changes for ΔE greater than 5 and ΔE greater than 10 was seen on test patches cleaned with Kodak Photo-Flo. This product was a poor performer at controlling bacteria in both sunny and shady locations in all cemeteries. It also ranked the lowest of all cleaners in limiting biological activity overall.

H₂Orange Grout Safe cleaner seemed to perform well based on color measurements and performance rankings of biological activity after six months. However, closer inspection of photographs taken from Jefferson Barracks National Cemetery indicated that biological staining was present on the edges of the headstone patches cleaned with H₂Orange Grout Safe cleaner. The location of the staining was not near the location of color measurements and was not reflected in the biological activity. Twelve months after the cleaning, all stains had disappeared. Researchers hypothesize that the H₂Orange Grout Safe cleaner did not kill all microbes initially and it took some time for all growth to die. Use of H₂Orange Grout Safe cleaner left an undesirable surface appearance for a period of time and thus was eliminated from the study.

Water and three cleaners remained – D/2 Antimicrobial cleaner, Daybreak cleaner, and World Monument Group Marble cleaner – as the project moved to phase two of the study. Appearance and biological activity continued to be documented for these cleaners in November/December 2006. Since further cleaners were being evaluated and the data from biological activity was more variable, few differences were noted. D/2 and Daybreak performed similarly in controlling overall biological activity. Researchers are concerned about possible chemical and physical changes from these cleaners, which are still under investigation.

Laboratory studies, including two accelerated weathering studies, were initiated. The first weathering study involved all six cleaners and two marble types. Lab test stones were cleaned on a daily basis for 33 days while being exposed to UV light, temperature cycles, and condensation cycles. These results were later considered to be too harsh, and a second accelerated weathering study was performed. In the second study, lab samples of Colorado Yule marble were cleaned and rinsed four times throughout the 33 day exposure.

Accelerated weathering samples are currently being evaluated for physical and chemical changes. Physical changes are being documented by changes in surface texture, color, and porosity. Chemical changes are being examined by optical microscopy, X-ray fluorescence spectroscopy, scanning electron microscopy, and determination of total soluble salts using gravimetric and conductivity methods. Accelerated weathering

samples cleaned with D/2 and Daybreak show some evidence of efflorescence which is being investigated.

Lab test samples were placed beside field test headstones during phase one of the study. They have been retrieved and were received at NCPTT on April 2, 2007. They will undergo evaluation similar to that described for accelerated weathering samples above. Phase two of the project will continue through fall 2007. A student intern will be assigned to assist in the analysis of lab and field test stones.

Researchers associated with the project, including scientists at NCPTT and biologists at the Laboratory of Applied Microbiology, Harvard University, are concerned than an eighteen month time period may not have been sufficient to document significant visual changes or to allow for the growth of algae and photosynthetic bacteria. The absence of algae and photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from stones treated with water, suggest it is still too early to determine the effectiveness of any biocidal properties of the cleaners.

While some results can be obtained by the expected completion date of October 2007, continuation of the study for two additional years is recommended. NCPTT staff present four possible options for continuation and the study in this report, and other options are available. It is advisable that some decision regarding extending the study be made prior to June 2007 (the date of the final field trip to the cemetery test sites).

2. Background

The Department of Veteran Affairs provides patient care and veteran's benefits – including burial-related entitlements – to 70 million veterans and eligible family members. An agency of the Department of Veteran Affairs, the National Cemetery Administration maintains 3.6 million occupied gravesites in its 120 national cemeteries and 33 soldiers lots, which total more than 14, 250 acres.

Visitors to national cemeteries expect to find the burial grounds well-cared-for and looked after. Part of this expectation is that the headstones are well-aligned and display a pristine, white appearance. These beliefs lead to relatively frequent cleaning of federally issued headstones, particularly compared to cleaning efforts undertaken in private cemeteries. Over time national cemetery staff and visitors have noticed a deterioration of stones from weathering. When headstones show significant loss of legibility or deteriorating conditions, the headstones are replaced.

One contributing factor to the weathering of stones may be the selection and use of chemical cleaners on a regular basis. C. Price notes that cleaning is one of the first steps in the conservation of stone and leads to improved appearance. However, cleaning with unsuitable cleaning methods can damage the stone by the loss of surface, staining,

deposition of soluble salts, or making the stone more vulnerable to pollution or biological growths.¹

Within the fields of conservation and historic preservation, guidelines for the care of cultural resources, such as cemetery headstones, have been established based on ethical considerations. First and foremost, a conservation treatment, such as cleaning, should do no harm. Staff and volunteers undertaking the cleaning should choose the gentlest and least invasive methods. Guidelines also recommend that those undertaking the work should not use chemicals without thorough understanding of how those chemicals react to the materials of the artifact and any material that may have been applied later.

On December 16, 2003, the National Cemetery Administration took the lead to organize an interagency task force to develop solutions to shared issues of interagency responsibility for historic government-provided headstones in an effort to supply consistent service to the American public in keeping with agency policy and mission. Topics included definitions of what is "historic," the science and technology of appropriate cleaning, and when to repair or replace. One outcome of this task force was the identification of the need for scientific research on cleaning methods for headstones.

Based on observations in national cemeteries, documentation in conservation literature, ethical considerations, and recommendations of the Interagency Task Force on Government-Issued Headstones, this research study was devised through collaborative efforts of the National Cemetery Administration and the National Center for Preservation Technology and Training.

2.1. Purpose of Study

On September 13, 2004, the National Cemetery Administration and the National Center for Preservation Technology and Training entered into an agreement to study the effectiveness of commercially available cleaners to remove biological growth from federally-issued headstones. The project goal was to test cleaning products for effectiveness and appropriateness and to make recommendations of products and methods best suited to both clean and preserve the headstones.

Cleaners in this study are evaluated based on multiple criteria to include:

- Appearance immediately after cleaning and over time,
- Physical changes to the stone, such as surface roughness or porosity,
- Chemical changes to the stone, such as chemical interactions with the cleaners or residual chemicals left on the stone,
- Biological activity after cleaning and over time, and

¹ Price, C.A., 1996, *Stone Conservation, an Overview of Current Research*. Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 7-14.

² Code of Ethics and Guidelines for Practice of the American Institute for Conservation of Historic and Artistic Works, revised 1994, Washington, DC: AIC. http://aic.stanford.edu/about/coredocs/coe/index.html ³ The Secretary of the Interior's Standards for Rehabilitation & Illustrated Guidelines for Applying the Standards (1992), National Park Service, Washington, DC. http://www.cr.nps.gov/hps/tps/tax/rhb/index.htm

• Ease of use and suitability for large-scale cleaning projects.

Main tasks associated with the project include both field and laboratory testing over a two-year period. The project is designed as a two phase project.

Phase one of the study includes the selection of cleaners and national cemetery test sites within five NCA regions or Memorial Service Networks. One aspect of the research looks at chemical cleaners that represent a variety of cleaning actions, for example basic versus acidic cleaning or ionic versus non-ionic cleaning. The research includes different geographic and climatic regions, such as a semi-tropical versus a dry or temperate climatic zone. Finally, cleaners are tested on two different types of marble – Colorado Yule marble from Marble, Colorado and White Cherokee marble from Tate, Georgia.

Five cleaning products are tested in side-by-side test patches on headstones in sunny and shady areas of each cemetery. Concurrent with the test patch studies, a series of cut marble samples are treated with each of the five products and exposed beside the test patch stones. These samples are used in both non-destructive and destructive laboratory testing. These laboratory samples help detect residual cleaning products on the stone and aide in evaluating potential stone deterioration.

Phase two of the study is based on results of the test patch evaluations after at least nine months of study. Based on phase one, three cleaning products are further tested on whole headstones. Whole stone studies allow for further evaluation based on visual appearance and ease of use.

3. Phase One of Study

Phase One of the study can be described in terms of planning and implementation and has distinct tasks associated with each activity. Planning activities included the choice of:

- Cleaners.
- Cemeteries,
- Headstones, and
- Evaluation methods for change in appearance and biological activity.

Implementation of the plan included

- visiting each cemetery,
- identifying and documenting headstones in sunny and shady locations within each cemetery,
- taking selected headstones out of regular maintenance cycles,
- making initial biological swabs for each headstone to establish baseline biological activity,
- making color measurements at each test patch to establish initial appearance,
- cleaning test patches on each headstone with each of the cleaners,
- monitoring the change in appearance through photographs and color measurements over time,
- monitoring biological re-growth through biological testing over time, and

• evaluating the data.

Phase One of the study began April 13, 2005 with a meeting of the partners held in the ASAE building at 1575 Eye Street, Washington, DC. Attendees at the meeting included Sarah Amy Leach, Karen Ashton, and Dave Schettler from NCA and Mary Striegel from NCPTT. Jason Church (NCPTT) and ElizaBeth Guin (Northwestern State University) participated by conference call. The purpose of the meeting was the selection of cleaning products and the identification of cemeteries to include in the study.

3.1. Choice of Cleaners

The most important variable in the study was the choice of cleaners. The team investigated fifteen possible cleaners for inclusion in the study. Possible cleaners are shown in Table 1. The chemical action of these cleaners includes acids, bases, alcohols, chelating agents, solvents, surfactants, and bactericides.

Acids and bases are strong chemical cleaners that work on the basis of the pH of the product. Products such as the Stone Kleen contain ammonium bifloride that easily converts to hydrofluoric acid, a strong acid that can chemically etch and potentially damage the surface of a stone. On the other end of the spectrum is the Kandu product, a low foaming cleaner containing sodium hydroxide, a basic compound.

Alcohols work on the basis of dissolving dirt and grease. They tend to evaporate quickly and are less likely to leave chemical residues. They may be one component of a multi-component cleaning system. Four of the products, including World Environmental group Marble Cleaner, incorporate alcohols into their formulas.

Chelating agents work on the premise that the cleaner will bind the dirt or grime to itself in order to remove the soiling. Some products containing chelating agents include Stone Quest, Zep-A-One, and World Environmental Group Multi Surface Cleaner 1000.

Solvents work similarly to alcohols in that they dissolve the soiling and may be a component of a more complex cleaning system. Only the product GK125 is listed as a solvent-based cleaner. Solvents are incorporated into some of the other cleaning systems.

Surfactants are wetting agents that lower the surface tension on a liquid allowing for easier spreading. Surfactants also allow grease and oils to be diluted and mixed into water and washed away. They are commonly found in cleaning detergents. Stone Quest, Multi Surface Cleaner 1000, Zep-A-One, World Environmental Group Marble Cleaner, D/2, Kodak Photo-Flo, and Kodak Hypo Clear all contain surfactants.

Bactericides are chemicals that kill bacteria and are commonly found in disinfectants, antiseptics, or antibiotics. One group of bactericides contain cationic surfactants such as quarternary ammonium cations. The D/2 product is an example of a bactericide containing quaternary ammonium cations. Another group of bactericides contain strong acids. Stone-Kleen is an example of a strong acid bactericide.

Product Name							
	acidic	basic	alcohol	chelate	solvent	surfactant	bactericide
Stone Quest							
Stone Care International				X		X	
GK125							
Geokleen Inc.					X		
Multi Surface Cleaner							
1000							
World Environmental							
Group, Inc.			X	Χ		X	
Omni-Green							
National Plastics and							
Chemical Corp.							
Stone-Kleen							
Mid Atlantic Chemical	X						Χ
H ₂ Orange ₂ Grout Safe							
Proven Solutions	Χ						
Hurricane Intensive Stone							
Cleaner							
National Chemical							
Laboratories			Х		Χ		
Zep-A-One							
Zep Manufacturing, Co.				Χ	Χ	Χ	
Marble Cleaner							
World Environmental							
Group, Inc		Χ	Х	Χ		Χ	
Kandu #110							
SpaceAge Coating							
Concepts, Inc.		Χ					
Daybreak							
NCH Corporation,							
Certified Labs		X					
D/2							
Sunshine Makers, Inc.						Χ	X
Sodium Bicarbonate							
Kodak Photo-Flo							
Kodak Corporation			Х		Х	X	
Hypo Clear							
Kodak Corporation				Χ		X	X

Table 1. A listing of chemical cleaners considered for testing, including main

The five cleaners chosen for inclusion in the study are shown in Table 2. The team wished to include cleaners that were environmentally friendly, user friendly, and were unlikely to damage the stone. Cleaners containing strong acids and bases, such as Stone-Kleen and Kandu, were eliminated on this basis. Daybreak was the most commonly used cleaner within the NCA, and thus was included in the study. H_2 Orange Grout Safe was chosen to represent an acidic cleaner containing citric acid. D/2 Antimicrobial cleaner was chosen as a bactericide and cleaner. The team felt that the two products by the

World Environmental Group were very similar in nature and relied on surfactants and chelating agents. The World Environmental Group Marble (WEG Marble Cleaner) was chosen for inclusion in the study. The final cleaner selected was the Kodak Photo-Flo because of its common use in the cemetery cleaning world. Table 3 shows the range of pH values found for the products chosen for the study.

	pН	Acidic	Basic	Alcohol	Chelate	Solvent	Surfactant	Bactericide
D-2	9.5						X	X
Daybreak H2Orange2 Grout Safe	12.1 3.81	X	X					
Kodak Photo-Flo	7			X		X	X	
Marble Cleaner	10.5		X	X	X		X	

Table 2. A listing of chemical cleaners chosen for the study, including published pH and component ingredients.

Cleaner	H2Orange2 Grout Safe	Kodak Photo-Flo	D-2	Marble Cleaner	Daybreak
pН	3.81	7	9.5	10.5	12.1

Table 3. Chosen Cleaners are ordered from Acidic to Basic.

3.2. Choice of Cemeteries

The second major variable in the study was the choice of locations for testing the cleaners. Did the biological growth found on headstones differ by location? Would bacteriai, algaes, or fungi dominate in some locations and not in others? How would climatic differences affect cleaning decisions? Would some cleaners perform better in some geographic areas and worse in others? To look at these issues, the team felt it was important to choose cemeteries that were geographically and climatically distinct.

Climate is the trends in weather patterns over an extended period of time. Two of the most important factors determining an area's climate are air temperature and precipitation. One way to classify climatic zones is using the Köppen Climate classification system. Within this system, five major climate types are classified based on average temperatures and precipitation, and designated by a capital letter. Subgroups are designated by a second, lower case letter which distinguish specific seasonal characteristics of temperature and precipitation. Further variations are noted by additional subgroups.⁴

In addition to climatic zones, NCA cemeteries are assigned to Memorial Service Networks (MSNs) based on their geographic location. The MSN offices are located in

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⁴ Köppen Climate Classification System, see http://en.wikipedia.org/wiki/Koppen_climate_classification.

Philadelphia (MSN 1), Atlanta (MSN 2), Denver (MSN 3), Indianapolis (MSN 4) and Oakland (MSN 5).

Cemetery	History	Climate Zone	Climate Description
San Francisco National Cemetery, San Francisco, CA MSN 5 – Oakland, CA	First burial: 1850 The site was formerly part of an military post established by the Spanish, continued by Mexico, and seized by the United States Forces during the Mexican War.	Zone Csb, using the Köppen Climate classification system, Mediterranean Climate	This region is characterized by temperate wet winters contrasting with warm or hot summers. The average annual rainfall is between 15 and 55 inches and occurs between November and April.
Santa Fe National Cemetery, Santa Fe, NM MSN 3 – Denver, CO	First burial:1868 Original interments are the remains of 265 United States Soldiers for the battlefields of Glorieta, Koslouskys, and the Old Fort March (General Kearney's Camp of 1847).	Zone Bsk, Semi-arid steppe climate	The steppe climate is characterized by hot summers and cold winters with 10 to 20 inches of rain or snowfall a year. It is similar to a praire.
Jefferson Barracks National Cemetery St. Louis, MO MSN 4 – Indianapolis, IN	First burial: 1827 The national cemetery included the old Post Cemetery containing burials made as early as 1827 from the Garrison of Jefferson barracks.	Zone Dfa, Humid continental	This region is characterized by a humid, cold climate with harsh winters and year-round precipitation.
Alexandria National Cemetery, Pineville, LA MSN 2 – Atlanta, GA	First burial: 1867 The cemetery contains burials from the civil war through the present.	Zone Cfa Humid Sub-tropical	This region is characterized as a mild climate with no dry season, and a hot summer
Bath National Cemetery, Bath, NY MSN 1 – Philadelphia, PA	First burial: 1879 The cemetery was originally a part of the New York State Soldiers and Sailors Home, which was established in 1877	Zone Dfb Humid continental	This region is characterized as a humid climate with severe winter, no dry season, and a warm summer.

Table 4. Cemeteries chosen for this study, assigned to a typical climatic zone.

Based on climatic and geographic distribution, five cemeteries were chosen for the study (see Table 4.) They include San Francisco National Cemetery, Santa Fe National

Cemetery, Jefferson Barracks National Cemetery, Alexandria National Cemetery, and Bath National Cemetery.

3.3. Selection of Headstones

The next step in the study was the selection of headstones from each cemetery to be included in the research. In this process, the team considered the following questions:

- 1) Will the type of marble make a difference in the removal or regrowth of microorganisms and biological activity?
- 2) Will localized environmental conditions such as sun or shade, or orientation in the cemetery affect the regrowth?
- 3) Are there seasonal effects for cleaning? For example, is it better to clean in the spring or fall?

There are three main stone types commonly used to create federally-issued headstones. These stone types include 1) Imperial or Royal Danby, a white or bluish white marble form Danby , VT; 2) White Cherokee, a white-grayish marble from Tate, Georgia; and 3)Colorado Yule, a white-creamy marble from Marble, CO. Of the three stone types, the White Cherokee is the most easily recognizable based on its color and large grain size. The Royal Danby and the Colorado Yule are less easily distinguished. The team recommended where possible that testing be performed on two types of stone in each cemetery. One set of tests should include the White Cherokee Georgia Marble. The second set of tests should include Royal Danby or Colorado Yule marble.

Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations. Thus, half of the White Cherokee Georgia Marble headstones included in the study should be located in predominantly shady locations while the other half should be located in predominantly sunny locations within each cemetery. The same criteria also applied to the second set of Royal Danby/Colorado Yule headstones.

Finally, in order to determine if seasons affected cleaning and biological regrowth, one set of headstones were cleaned in the spring and on set of headstones were cleaned in the fall.

Once the testing criteria were established, Sarah Amy Leach and Karen Ashton contacted each cemetery director and informed them about the testing program in June 2005. They created a one page briefing sheet and an informational Q&A document for the project in order to educate VA staff and visitors to the cemetery about the study. Additionally, informational signs were installed at each cemetery.



Figure 1. Example of the informational sign placed in each cemetery for the duration of the study.

3.4. Evaluation Methods for Change in Appearance and Biological Activity
The emphasis during phase one was on the visual appearance of the stone before and after cleaning and on the amount of biological re-growth over time. The five cleaners were evaluated based on patches tests on stones in sunny and shady locations within each cemetery. These evaluations were used to eliminate or retain cleaners for phase two of the study.

3.4.1. Visual Appearance

The appearance of the headstone is defined as the outward or visual aspect of the stone and is considered a subjective judgment of the viewer. Since the appearance of the stone is particular to a given individual, it is influenced by cultural bias. In general, military headstones are expected to be clean, white, legible, and well-aligned. Thus unacceptable appearance includes gray, yellow, black, or mottled coloring from either biological growth, dirt, or chemical changes. In all phases of the study, two methods for documentation and analysis of appearance are proposed – use of photo-documentation coupled with visual ranking and color measurement.

3.4.1.1. Photodocumentation/Visual Ranking

Photography is the way information in the form of light documented from a subject, in this case a headstone. It is an easy way to document information from a site or location and covey it to others. However, the environmental variables and instrumental variables can affect the way the light is captured in the photograph. Thus, when documenting the headstones and cleaning patches with photography, some variability in the images will be due to the time of day in which the photograph was taken, and the camera settings (e.g. aperture, focal length, shutter speed, etc.). Also, the way in which the photograph is perceived varies from viewer to viewer.

Because of these considerations, photo documentation is a qualitative method for studying appearance. One way to deal with this qualitative information is to have viewers rank what they see in photographs based on a set scale. The viewers should know little about the subject prior to the ranking, making them unbiased viewers. This produces a way to evaluate the information recorded in a photograph which is semi-quantitative.

3.4.1.2. Color Measurement

Being able to quantify the color and surface appearance of stones is a crucial factor in this study. Color is a physiological process by which the human eye translates electromagnetic radiation. It is generally dependent on the observer, the object, and the environment in which the object is viewed.

A colorimeter is an instrument that measures red, blue, and green color components of light and is used to determine a specific color reflected from a surface. The color is specified in numeric terms using the CIELAB color system. Colors are specified in terms of L*, a*, and b*. The L* values represent lightness and can range from 0 to 100, with 0 designating black, and 100 designating white. The a* values represent the red-green chromatic component. Values of a* range from -100, designating green to 100, designating red. The b* values represent the yellow-blue chromatic component, with values ranging from -100 to 100. A pure yellow is represented by 100 and a pure blue is represented as -100 on the B* scale.

The CIELab system lends itself well to measuring change sin color over time. The total color difference, ΔE^* , can be calculated from:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

 ΔL^* is the lightness value difference between color 1 and color 2, = L^*_{1} - L^*_{2} Δa^* is the red-green value difference between color 1 and color 2, = a^*_{1} - a^*_{2} Δb^* is the yellow-blue value difference between color 1 and color 2, = b^*_{1} - b^*_{2}

Equation 1. The total color difference between two Lab color measurements.

A total color difference of less than $2 \Delta E^*$ is imperceptible to the human eye.

Color measurements of L*, a*, and b* are taken of the headstones prior to cleaning and documented. Since the surface of the stone is not completely smooth, three measurements are taken at each location then averaged. Measurements are repeated at each cleaning test site on regular intervals throughout the study.

3.4.1.3. Biological Testing

A general overview of the biological testing is presented here. Details of the biological testing can be found in Appendix D, Appendix E, and Appendix F. The team determined the biological testing scheme for the study in consultation with Dr. Ralph Mitchell, Department of Engineering and Applied Science, Harvard University. Initially, NCPTT

scientists proposed the identification of biological species present on a large number of headstones. However the actual number of samples to be taken and the time and effort to complete the biological analyses would have resulted in over 63,000 hours of work and was dismissed as untenable. Mitchell recommended general identification of bacteria, fungi, and photosynthetic microorganisms (algae) found on headstone prior to cleaning. Then, over time, counts of bacteria, fungi, and algae would be determined for test patches each cleaner in sunny and shady locations.

To determine the baseline biological activity, swabs are taken from a three cm² area of each test patch using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Bacteria and fungi are enumerated by plating samples on solid media. Plates are incubated at room temperature for two days and colonies are counted. Photosynthetic microorganisms (algae) are analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view are counted at 40X magnification.

3.5. Field Test Trials on Headstones

Once the planning activities of phase one were complete, implementation tasks were begun. Jason Church initiated the first of a field trip series beginning in June 2005⁵ to each of the test cemeteries. The purpose of these first trips was to initiate contact with each cemetery staff, to identify headstones for inclusion, to set field test samples for phase two of the study, and to take overview photographs of each cemetery for the study.

All stones selected for inclusion in phase one were taped in a grid system that created six test patch sites.

3.5.1.Documentation of Headstones

Headstones were photographed before cleaning and at six month intervals after cleaning throughout phase one of the study. All photographs were taken digitally and saved in JPEG format. Photographs were taken in October 2005, April 2006, and November 2006.

In October 2005, photographs were taken using a Sony DSC-S85 digital camera. The camera has a built-in 34 mm-102 mm zoom lens. All images were taken at 2272 x 1704 pixel resolution on auto-exposure and auto-focus settings.

All subsequent photographs of headstones, taken in April 2006 and November 2006, were taken with a Nikon D50 digital camera body fitted with an AF-S 18-55 mm zoom lens. Images were captured at 3008 x 2000 pixel resolution (Large, JPEG Fine) at an approximate 45 mm lens focal length.

Appendix A, *Photographic Documentation of Field Trials*, contains a series of photographs taken in six month time intervals each headstone in phase one of the study. An overview shot and details of each test patch are found for each headstone prior to cleaning and every six months as the study progressed.

⁵ Phase one field trips included Jason Church's site visits on June 5-11, 2005 to San Francisco CA, Santa Fe NM, St. Louis MO, and Bath NY. He visited Alexandria LA on April 25, 2005 and June 22, 2005.



Figure 2. Jason Church positions the head of the Minolta colorimeter for measurements on a headstone in Alexandria National Cemetery.

Upon completion of the photo-documentation, color measurements were taken using a Minolta Colorimeter, CR-400. Each measurement was repeated three times on each stone sample and averaged in order to compensate for slight variations in surface texture. Color measurements were consistently taken at the same location—the lowest point on the inside corner—within each grid (see Illustration 1).

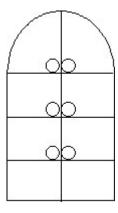


Illustration 1. The circles indicate the location where color measurements were taken within each test grid.

Appendix B, Color *Measurements on Field Trials*, provides measurement data for all color measurements taken on headstones in the field.

3.5.2. Initial Biological Activity

Initial biological activities were determined by culturing swabs taken from selected headstones within each cemetery. The purpose of these analyses was to establish the level of biological activity prior to any cleaning performed in this study.



Samples were collected by Jason Church from the five chosen cemeteries. Within each cemetery, samples were collected from 20 locations. A three cm² area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Samples were shipped overnight to Harvard University.

Figure 3. Photographic detail showing an acetate template being used as a guide for swabbing the headstone.

Results from this study are found in Appendix D. Analysis of Microorganisms on headstones in VA Cemeteries,

First Report: December 2005 and are summarized here. Bacteria and/or fungi were found in most samples in all five cemeteries. Algae, which are photosynthetic organisms capable of darkening or staining the headstones, were not found in samples taken during this phase of the study. The decreasing order of biological activities was:

Santa Fe > Jefferson Barracks > Alexandria > San Francisco > Bath

Santa Fe National Cemetery displayed the largest amount of bacterial and fungal activity of the five cemeteries, which was five times greater than any other location. Jefferson Barracks results showed small quantities of fungal growth on all but one headstone. Fungi were found on headstones in both sunny and shady locations. Bacterial counts were limited to a few headstones in Jefferson Barracks. In Alexandria, more bacterial and fungal activity was seen on headstones in shady locations over sunny locations. Bacteria were not detected in many samples from San Francisco National Cemetery, but when found, were more likely to be seen in sunny locations. In contrast, bacteria and fungi were detected in few samples from Bath National Cemetery.

Initially, the presence of higher biological activity at Santa Fe National Cemetery seemed counter-intuitive. Santa Fe is a drier climate and little biological soiling had been observed in the cemetery. Locations such as Jefferson Barracks or Alexandria would be expected to have richer environments for biological growth due to their climates and higher relative humidities. Additionally, a visual survey of private cemeteries in these regions showed typical biological growth, see Figure 4.



Figure 4. Example of biological growth found on a grave marker in the Jewish Cemetery, Pineville, Louisiana which is located four blocks from Alexandria National Cemetery.

It is important to note before evaluating results from initial biological analyses that each cemetery has its own regular maintenance schedule which will

influence the nature of the biological activity on headstones from that cemetery. Jason Church documented the cleaning activities of each cemetery by interviewing staff and maintenance crews (see Table 5).

Cemetery	Cleaner Used	Periodic Schedule	Methods
Santa Fe National	Zep Ring Master All	Spot cleaning as	Applied with
Cemetery	Purpose Bathroom	needed	portable sprayer and
	Cleaner		rinsed thoroughly
Jefferson Barracks	50% Clorox and	Annually	Applied with pump
National Cemetery	50% water		sprayer, or
			Backpack sprayer.
			Left un-rinsed.
Bath National	50% Clorox and	Annually, with spot	Applied with pump
Cemetery	50% water	cleaning as	sprayer, or
		necessary	Backpack sprayer.
			Left un-rinsed.
San Francisco	40% Clorox	Total cleaning once	Applied with
National Cemetery	Outdoor and 60%	a year with pressure	portable sprayer.
	water	washing as needed	Left un-rinsed.
Alexandria National	HTH Granular,	Annually, with spot	Applied with
Cemetery	mixed with water to	cleaning usually 8	portable sprayer.
	an unknown	months after	Left un-rinsed.
	concentration		

Table 5. Cleaning schedules and use for Santa Fe, Jefferson Barricks, Bath, San Francisco, and Alexandria National Cemeteries.

Santa Fe National Cemetery cleans headstones infrequently using a highly acidic product, Zep Ring Master Bathroom Cleaner⁶ for spot cleaning. Since the cleaner is a green liquid Santa Fe maintenance workers rinse thoroughly after cleaning. Jefferson Barracks National Cemetery cleans headstones annually using a 50/50 mixture of Clorox and water. The cleaner is applied with a backpack sprayer and left un-rinsed. Bath National Cemetery follows a similar cleaning regiment, cleaning once a year with the 50/50 Clorox mixture and following with spot cleaning as necessary. The cleaner is applied by sprayer and not rinsed after cleaning. A 40/60 mixture of Clorox Outdoor and water is used by the San Francisco National Cemetery to clean headstones using a portable sprayer. Headstones are not rinsed after cleaning. Alexandria National Cemetery uses HTH Granular, a calcium hypochlorite product commonly used for swimming pool treatments, to clean headstones.

Upon closer consideration of the data and the cyclic maintenance undertaken at each cemetery, logical conclusions could be drawn. This study did not begin with sterile stones inoculated with similar bacteria, fungi, and algae. The biological activity is a complex system influenced by seasonal changes, a variety of biota, the nature of the stone, and the history of headstone cleaning at each cemetery.

Santa Fe National Cemetery staff rarely cleans its headstones and then they undertake only spot cleaning as needed. Thus, a rich bio-film has developed over time on headstones in Santa Fe. Despite this biofilm, the stones appear clean because there is a lack of algae – the photosynthesizing organisms that can produce staining – or low numbers fungi.

In contrast, those cemeteries whose environments are likely to promote biological growth, such as Alexandria National Cemetery or Jefferson Barracks National Cemetery, are cleaned much more frequently in order to keep the stones white. In these places, HTH Granular (calcium hypochlorite) or bleach (sodium hypochlorite) is used for cleaning and left on the surface. After several cleaning cycles, the stones show much less biological activity.

The following general conclusions can be drawn:

- Bacteria and/or fungi were found in most samples.
- Numbers of bacteria were generally greater than numbers of fungi.
- Algae were not detected in the samples.
- Analysis of microbial growth showed wide variability in the size of the microbial community.
- Numbers of bacteria and fungi were low in most samples and may be due to the historical cleaning cycles the stone has seen.
- The presence of high numbers of bacteria and fungi at Santa Fe National Cemetery is likely due to its infrequent cleaning.

⁶ According to the published Materials Data Safety Sheet, Zep Ring Master has a pH of less than 1.0 and contains phosphoric, hydrochloric, and sulfuric acids (which can cause sugaring and loss of binder in marbles and limestones.)

3.5.3. Cleaning Headstones

Jason Church began cleaning headstones after (1) documenting their visual appearance using digital photography and colorimetry and (2) sampling their surfaces for biological activity.



Figure 5. Jason Church applies WEG Marble Cleaner to a taped test patch. He holds a piece of acetate to the stone surface to prevent runoff below the patch. All cleaners were applied following manufacturer's recommendations for dwell time, etc. The stones were subsequently rinsed with water, again using the acetate to prevent runoff.

A grid was taped onto each stone using one inch wide 3M blue tape. Cleaners were applied to the surface of the headstone following manufacturers' recommendations. An 8.5 x 11 inch acetate sheet was used to insure that cleaner did not run to a second test grid. If the stone had raised or engraved lettering, the acetate was taped to the irregular surface. Cleaners were applied in the same test grid on each stone, as shown in Illustration 1 After each test patch was cleaned, the area was thoroughly rinsed with tap water.

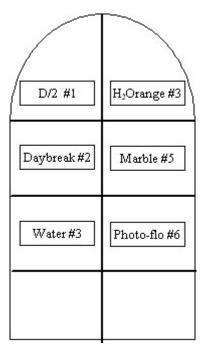


Illustration 2. This illustration shows the position of each cleaner on a headstone.

3.5.4. Appearance Changes

Based on visual observations, all cleaners effectively removed soiling and biological growth from the stone. Water removed soiling and to a much lesser extent staining from micro-organisms. Changes in appearance were recorded by photographs and color measurements. In general, there was natural variability in the results. NCPTT staff evaluated color change and visual appearance between April 2006 and November 2006, representing changes from six months to twelve months after cleaning. Most changes in appearance during this time were subtle.

Changes in color measurements were calculated from CIElab coordinates as ΔL^* (changes in lightness or darkness), Δa^* (changes towards red or green), Δb^* (changes towards blue or yellow), and ΔE (total Color change). These results are reported in Appendix B. *Color Measurements on Field Trials*.

Further evaluation of the data focused on establishing color change trends by counting the frequency of color changes at ΔE greater than 5, and ΔE greater than 10. Again, any changes of color observed were subtle to the human eye. Researchers looked at frequency trends compared by cemetery, by cleaners, and by sunny or shady location. The results are reported in Appendix C. *Color Analyses by Cemetery, Test Patch, and Location.* Once the frequency tables were created, photographs of each headstone were carefully examined to determine if measured color changes could be observed in the photographs. Two important points should be noted about the data. First, additional data regarding color changes will be measured in forthcoming field trips, thus data for some headstones continues to be collected. Second, some data from Bath National Cemetery is missing. These analyses exclude data from Bath National Cemetery at this time.

By looking at the frequency of ΔE color changes, researchers were able to initially identify headstones that displayed some subtle appearance changes. For example, data from headstones in Jefferson Barracks is shown in Table 6.

	Delta E for Jeff Barracks	erson				
Patch #	32 2904-A	32 2898-A	72 1273	72 1370	Freq dE> 5	Freq dE> 10
1	3.25	1.36	2.90	14.05	1.00	1.00
2	1.09	0.87	3.79	3.75	0.00	0.00
3	3.44	1.44	7.83	9.44	2.00	0.00
4	3.39	15.31	2.59	8.89	2.00	1.00
5	2.42	3.98	12.92	6.53	2.00	1.00
6	5.16	1.07	8.98	11.37	3.00	1.00
					10.00	4.00

Table 6. Frequency of color change greater than 5 (in yellow) and color change greater than 10 (in gold) for headstones at Jefferson Barracks National Cemetery, St. Louis, Mo.

By looking at this data, headstones 72 1273 and 72 1370 were identified as displaying color changes from April 2006 to November 2006. The same analyses were undertaken for data found in Appendix C from each cemetery. Based on color measurement changes

headstones at Jefferson Barracks displayed the most occurrences of color change in patch 6 (Kodak Photo-Flo). Next, researchers closely inspected photographs of headstones that displayed color changes to see if further visual changes could be noted. For example, upon closer inspection of headstone 72-1273, biological re-growth was noted in patch 4 (H₂Orange Cleaner) along the inset center cross, see Figure 6. Further review of the color measurement data indicated that the stone had darkened slightly (based on negative ΔL^* values for all patches).

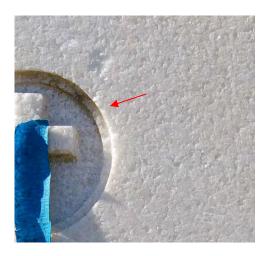


Figure 6. Close-up detail of headstone 72 1273, showing signs of biological growth on inset.

On closer inspection of headstone 72-1370 in Jefferson Barracks, scientists noted a mottling appearance that could be seen in most patches, see Figure 7. The headstone is darkening. There may be biological re-growth associated with the veining seen in the stone.





Figure 7. An overview, left, and a detail of patch 4, above, showing the mottled appearance on headstone 72-1370, Jefferson Bararacks. Photograph taken November 2006.

Similar observations can be made at other cemeteries. For example, color change was noted in several headstones in San Francisco, including WS 1032 B shown in Figure 8. Based on color measurements, five of the six test patches have changed color by ΔE greater that 5. Most of this color change comes from the darkening of the headstone (negative ΔL^* values). Visual observation supports these measurements. Biological growth can be seen on patch 2 (D2 cleaner) patch 4 (H2Orange cleaner) and patch 5 (WEG marble cleaner). Also, the brown staining seen on the lower portion of this stone is frequently found in the cemetery. Researchers hypothesize that the staining is due to the use of iron fortified fertilizer used by contractors in San Francisco National Cemetery.





Figure 8. Overview, left, of WS 1032B, San Francisco National Cemetery taken November 2006. Biological re-growth is evidenced on the stone, particularly in patches 2, 4 (above) and 6 (below).



Next, frequency data of ΔE color change was analyzed by cleaner, see Appendix C. The frequency of color change is given in Table 7.

Cleaner	Freq dE> 5	Freq dE> 10
D/2	5.00	1.00
Daybreak	7.00	2.00
Water	7.00	2.00
H2Orange Cleaner	5.00	2.00
WEG Marble Cleaner	8.00	1.00
Kodak Photo-flo	11.00	3.00

Table 7. This table shows the number of color changes greater than 5 and greater than 10 for each cleaner found on headstones at Alexandria, Jefferson Barracks, San Francisco, and Santa Fe National Cemeteries.

From this data, Kodak Photo-flo exhibited the greatest number of color changes both greater than 5 and 10. Based on this frequency analysis, the worst performer was likely Kodak Photo-Flo. Although Photo-flo test patches, location #6 (Illustration 2), were lower on the headstones it is unlikely that these changes were a result of rain water backsplash since water, in adjacent location #3, did not show the same frequency trend.

Finally, frequency data of ΔE color change was analyzed based on the location of the headstone in sunny or shady locations within the cemetery. The frequency analysis of this data is given in Table 8. The frequency of color changes greater than 5 is equal in sunny and shady locations (n = 22), indicating that there is a equal chance of seeing a color change in a sunny location or a shady location on the headstones. However, there is a greater chance of seeing a color change greater than 10 in a shady location than in a sunny location. Fungi and algae tend to grow in shady locations and may lead to greater visual appearance changes.

Shady		
Cleaner	Freq dE> 5	Freq dE> 10
D/2	2.00	0.00
Daybreak	4.00	2.00
Water	3.00	2.00
H2Orange Cleaner	3.00	1.00
WEG Marble Cleaner	4.00	1.00
Kodak Photo-flo	6.00	2.00
	22.00	8.00

Sunny		
Cleaner	Freq dE> 5	Freq dE> 10
D/2	3.00	1.00
Daybreak	3.00	0.00
Water	4.00	0.00
H2Orange Cleaner	3.00	1.00
WEG Marble Cleane	4.00	0.00
Kodak Photo-flo	5.00	1.00
	22.00	3.00

Table 8. This table shows the number of color changes greater than 5 and greater than 10 headstones in sunny locations and shady locations at Alexandria, Jefferson Barracks, San Francisco, and Santa Fe National Cemeteries.

Some unusual visual observations were made at Jefferson Barracks National Cemetery. It was at this cemetery that Church inadvertently cleaned more stones than were needed for the study. In follow-up visits, biological activity was observed on these headstones as

well as headstones included in the study. Eight headstones were cleaned at Jefferson Barracks in October 2005 – six headstones were in the shade and two headstones were in the sun. On all shady headstones, the reoccurrence of biological growth was seen predominantly on patches cleaned with H₂Orange Cleaner (#4). The re-growth was sometimes seen on other patches #5 and #6 below the H₂Orange Cleaner. Figure 9 shows examples of biological re-growth on four of the stones. Sample and swabs and biological analysis did not indicate any significant differences between patch 4, cleaned with H₂Orange cleaner, and other test patches. Interestingly, six months after these photographs were taken the dark biological growth had disappeared on all samples! It may be possible that H₂Orange cleaner did not kill all microbes initially, and it took some time for all growth to die. Alternately, the growth seen on these headstones was seasonal and may return again in the future. In any case, the reoccurrence of microbial activity left an undesirable surface appearance for a period of time, thus NCPTT staff recommended the exclusion of H₂Orange cleaner from Phase Two of the study.

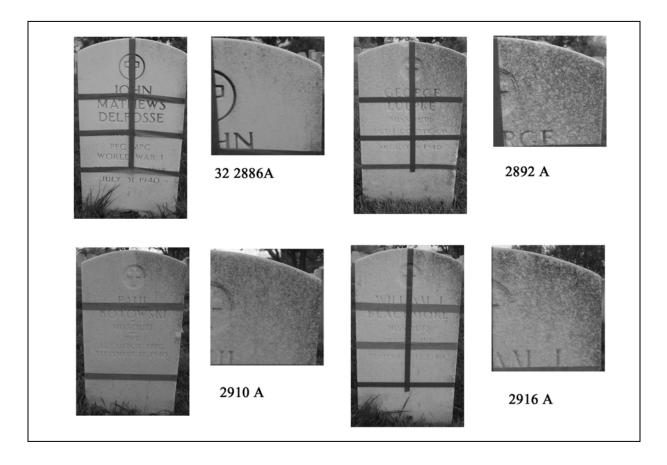


Figure 9. Overview and detail photographs of four headstones found in shady locations of Jefferson Barracks National Cemetery are shown. Microbial activity is found predominantly on patches cleaned with H₂Orange cleaner.

3.5.5.Follow-up Biological Activity, June 2006 Report
Headstones that were cleaned in October 2005 were swabbed again in April 2006 to
determine biological activity. These results are reported in Appendix E. Analysis of

Microorganisms on Headstones in VA Cemeteries,

Second Report: June 2006 and are summarized here. The work here involved looking at many of the samples, but only a select number of samples were enumerated in this round of analyses.

No algae were detected in samples from any of the five cemeteries. Green coloration in some samples was due to the presence of fungi. In general, the numbers of bacteria were greater than the numbers of fungi found on the stones.

The decreasing order of biological activities was:

Bath > Jefferson Barracks > San Francisco > Alexandria > Santa Fe

Bath had five times more enumerated bacteria than bacteria found in Santa Fe, but both counts are in the same order of magnitude. In Santa Fe, the biological growth of both bacteria and fungi is greater in shady areas. Higher fungal counts were found in Bath and Jefferson Barracks. At Jefferson Barracks, fungal growth was higher in the shade than in sunny areas. Lower biological activity in Santa Fe may be expected because of the drier, hotter climate.

The enumerated biological activity does not fully account for visual changes observed on headstones. This is partly due to the fact that only one stone displaying discoloration, from Jefferson Barracks, was enumerated in the biological activity study. Also, the biological analyses did not attempt to identify specific fungi or bacteria genus present. One hypothesis is that some cleaners are not full spectrum and thus don't fully kill all fungi or bacteria. If most micro-organisms are eradicated, but a few are left behind, then those left behind may grow freely. Resistant fungi that continue to grow may cause discoloration but be limited to a select species.

Further analysis of the biological activity regarding biocidal effectiveness of each cleaner becomes more complex. NCPTT researchers chose to rank the biological activity for each cleaner to determine performance.

3.5.6. Ranking Biocidal Performance of Cleaners

Dr. Tye Botting prepared a ranking of the performance of the cleaners to inhibit biological growth based on the enumerated microbial activities determined from the June report. Botting ranked activities from 1 to 6 with lower numbers indicating higher biological activity found in the count. He ranked the activity by cemetery site, sunny or shady location, bacterial count, fungal count, and cleaner. Once her ranked each cleaner, he averaged the results for overall activity in sunny locations, overall activity in shady locations, and by total growth. He also looked only at bacterial activity and fungal activity. Results are found in Appendix G. *Biological Performance Based on June 2006 Report*. In this appendix, Botting highlighted those cleaners with average rankings below 3.0. The following observations can be made based on the rankings:

- Photo-flo was a poor performer at controlling bacteria in both sunny and shady locations at all cemetery sites. It also ranked lowest of all cleaners in limiting biological activity overall.
- D2 performed well in controlling bacteria and fungi in sunny locations and in controlling bacteria in the shade. Greater activity of fungi was seen on test patches cleaned with D2 in shady locations.
- D2 and Daybreak performed similarly in controlling overall biological activity.
- WEG Marble cleaner was a consistently high performer based on this analysis.

3.5.7. Follow-up Biological Activity, January 2007

A second analysis of biological activity focused on headstones that had been previously swabbed in October 2005 and April 2006. Swabs were collected at the designated cemeteries in October/November 2006. Swabs of patches cleaned with D2, Daybreak, WEG Marble Cleaner, and water were analyzed for biological activity, and they are reported in Appendix F.

Trends in biological re-growth were not evident in the evaluation of this data. No consistent differences were found for bacteria or fungi for the remaining cleaners. In general, numbers of bacteria were greater than fungi at all cemeteries. The greatest difference was seen between sunny and shady locations. Greater numbers of bacteria and fungi were found in shady areas. This is most likely due to drier conditions and more intense UV irradiation in sunny locations.

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.

Further ranking of this data, similar to the performance rankings discussed in Section 3.5.6, is shown in Appendix H. In this ranking, the performance of D2, Daybreak, WEG Marble Cleaner, was evaluated based on the numbers of bacteria, fungi, or total re-growth enumerated approximately twelve months after cleaning. Rankings were from one to four, with higher numbers indicating better performance. Ties were allowed. Few trends in biological activity were identified based on this analysis. Cleaners performed about equally in the shady areas. Water was the worst performer in slowing bacterial re-growth in shady areas. WEG Marble Cleaner was a poor performer for slowing bacterial regrowth in sunny locations.

Thus, all indications from the biological activity analysis is that more time is needed to allow significant re-growth to better distinguish between cleaners.

4. Phase Two of the Study

The primary concern in phase one was the biological aspects of the cleaners being tested. The end of phase one was concluded with the removal of two cleaners due to their poor biocidal properties. Phase two is primarily concerned with testing to see if the remaining cleaners have any adverse physical or chemical effects on the marble. This will be tested in three studies; the first will be on whole headstones cleaned in the field, the second is testing on the sample stones that were treated and weathered in the cemeteries and the third is a the continuation of the accelerated weathering studies done at NCPTT's laboratories.

4.1. Laboratory Testing

Laboratory testing in phase two of the study evaluated two groups of samples. The first group to be tested is laboratory samples that were treated with the select cleaners while going through an accelerated weathering phase in a QUV weatherometer. The second group to be tested is sample stones that were treated and weathered in the select cemeteries for the past 18 months. These stones were recently removed from the cemeteries and sent to NCPTT for testing.

Samples from both the laboratory study and the field test will undergo similar treatments. The first series of tests will look for any severe deterioration of the stones structure (such as surface loss) or discoloration of the surface. This includes photographic comparison, colorimetery measurements and laser profilometry among others. The next series of tests will look for soluble salts or other chemical residues left on the marble due to the cleaners. The marble will undergo a series of destructive and non-destructive testing. Preliminary destructive methods of testing for soluble salts include electrical conductivity and various gravimetric methods. The presents of soluble salts inside the marble changes the stone's pore structure. This change has negative effects on the way the stone will weather overtime. Methods used to test the two marble types for pore change include; mercury intrusion porosimetry and nitrogen absorption porosimetry. X-ray diffraction and XRF spectrometry will also me performance on the samples to help determine any chemical contamination to the stones. The level of testing will be determined by the amount of information found in the preliminary tests.

4.2 Whole Headstone Cleaning

Phase two of the study began by cleaning whole headstones in each of the five test cemeteries. In the fall of 2006 Jason Church traveled to each of the sites beginning with Bath National Cemetery on November 7th and ending with Alexandria National Cemetery on January 16th.

To begin this phase of the study, the remaining three cleaners – D/2, Daybreak and WEG Marble Cleaner – were used evenly to clean a total of 24 whole headstones in each cemetery. Of the 24 markers half are Colorado Yule marble and the remaining half are Georgia marble. For comparison purposes half of the stones were sprayed with the cleaners and the other half was physically agitated. Before any cleaning was done the headstones were first photographed and colorimetry measurements were taken. This information will be used to compare the stones appearance over time.

When whole headstones were cleaned, each of the manufactures recommendations were followed. Headstones were always cleaned from the bottom to the top starting with the face and proceeding around the stone counter clockwise. After the cleaner was applied and had significant dwell time, headstones were rinsed thoroughly with water from the site. In the cases where headstones were only sprayed with the cleaner, a Cepia 1-touch motorized sprayer was used. This 32oz handheld powered sprayed helped to control the amount of cleaner used and regulated the force in which the cleaner was applied. Rremaining headstones were cleaned using agitation. The cleaner was first applied to the stone using the motorized sprayer. Then the cleaner was agitated in a small circular motion starting from the bottom and working up using a soft natural bristle brush that measures approximately 3" by 9". After the surface of the stone had been evenly scrubbed the entire stone was rinsed.

During the next round of cemetery visits which will begin in May of 2007 photographs and color measurements of the cleaned headstones will be taken. These will be used to compare any change over time. Also, during this visit a measurement will be taken of each of the headstone using the portable XRF spectrometer. This will help determine if any of the cleaners left behind residual chemicals.

5. Current Research Activities

NCPTT staff is currently undertaking phase two research activities that focus on understanding physical and chemical changes to the stone. These efforts include laboratory studies involving accelerated weathering and comparison of accelerated results will field experiments that have been undertaken simultaneously.

5.1. Laboratory Studies

Laboratory studies in phase two consist of accelerated weathering studies at NCPTT laboratories and analytical evaluation of the laboratory samples and field test samples placed in the five chosen cemetery sites.

5.1.1. Accelerated Weathering Studies

Figure 10. Georgette Lang cores a sample of Colorado Yule for use in the accelerated weathering study.

ADELTA

In June of 2006 NCPTT began the first of two accelerated weathering studies. The purpose of these studies was to

simulate the long term use and exposure of the five selected cleaners on two types of marble. Newly quarried Colorado Yule marble and Cherokee White Georgia marble were obtained from the NCA contracted quarries. "New" marble was selected for these studies so that any residual chemicals found on the stone after the accelerated study could be attributed to the cleaner used and not to any prior treatments on the marble. By doing a laboratory accelerated weathering experiment; factors could be controlled such as humidity and light and dark exposures. Thus the samples and cleaners were compared under the same controlled conditions.

All accelerated weathering studies used a Q- Panel Lab Products model QUV/ Spray Accelerated Weather Tester (weatherometer). This instrument uses panels of UVA-340 lamps to control a programmable cycle of light and dark. The bulbs irradiance level is calibrated to a constant level of 0.77 W/m2.



Figure 11. Marble samples being removed from the QUV weatherometer during a dark cycle to be treated with cleaner.

For both accelerated studies, the Weatherometer was programmed for a continuing cycle of UV exposure for 4 hours at 60 degrees C followed by 4 hours of condensation at 50 degrees C. Note that this step was in the dark (no UV light) to mimic the natural cycle of night and day, and the temperature drop encouraged condensation from the surrounding humid air inside the Weatherometer. The water that condensed inside the Weatherometer initially comes from a lower holding pan that was supplied from a filtered water system that generated 18 megohm-cm purity of water. These cycles repeat for a total of 800 hours.

Marble samples were prepared in the same manner for both accelerated weathering studies. Newly quarried marble was placed on a drill press and cored with a water jacketed diamond coring bit to a diameter of 1 5/8 inches. Then cores were sliced with a water cooled MK tile saw to a uniformed thickness of ½ inch. Once all of the samples were cut to size, they were placed on a Buhler Ecomet 4 fitted with an Automet 3 rotating head and polished to remove any remaining saw marks. The Colorado samples were polished for 5 minutes at 30 rpm with 7 lbs. of force using a 120 grit sanding disk. The Georgia samples were polished for 5 minutes at 30 rpm with 12 lbs. of force using 120 grit. These steps were then repeated using 220 grit paper.

5.1.1.1. Thirty-Three Day Cleaning Study

The first accelerated weathering study began on July 24, 2006. This study was conducted by Georgette Lang, a chemistry major at Centenary College of Shreveport, Louisiana under the supervision of Jason Church. For this study two types of marble were cored and prepared. Three replicate samples of marble were prepared for each type of cleaner. Along with these samples, three untreated samples of each marble type were readied as internal standards. Finally, three samples of each marble were prepared that would not be treated in the Weatherometer but remained untreated as control samples. This brought the total number to 48 samples. Each sample was given a unique number which encoded information about marble type, chemical cleaner used and the sample identification number. This unique 3 digit number was inscribed on the back of each sample.

Pre-existing conditions of the marble surface were recorded by using the Laser Profilometer (see section 4.3.2.1.1.) to map the surface of each sample prior to treatment. Each sample was photographed and color measurements were taken to check for any color change as a result of the application of the cleaners. The weight of each sample was recorded as a baseline to identify residual material deposited from the cleaning. Once the samples were documented and mounted into the Weatherometer sample holders, the 800 hour test was initiated.

The samples were sprayed with the select cleaner and rotated inside the Weatherometer on a daily bases. The marble was treated with the six cleaners D2, Daybreak, Kodak Photo-flo, H2Orange2, Marble Cleaner, and water (plus one set that was weathered but untreated). Each of the chemicals was mixed to the manufactures recommendation and applied to the sample using a 16 oz hand pump spray bottle. The samples were removed from the Weatherometer in their holder and sprayed to completely wet the surface at the end of a dark cycle at approximately the same time each day. The end of a dark cycle was chosen as a time for treatment so that the cleaner would have sufficient time to soak into the stone without evaporating at elevated temperatures during the UV exposure. After the sample was sprayed, it was placed back into the Weatherometer without being rinsed. The decision was made not to rinse the marble after it had been treated because 4 out of the 5 cemeteries involved in the study stated that they do not rinse their stones post cleaning.

On August 27, 2006 the Weatherometer run ended and the samples were left in the powered down Weatherometer for 48 hours to allow any moisture in the stone to evaporate. Testing began after the samples were removed from their holders. Once accelerated weathering was concluded, testing repeated using the same methods as pretesting documentation. First, the weight measurement is taken. Second, samples were photographed, and third, colorimetry measurements were taken. Finally, surface texture on each sample was measured using the laser profilometry. Also, at this time each of the elemental composition of sample surfaces were analyzed using the Tracer III portable X-Ray Fluorescence Spectrometer. The XRF Spectrometer under the following conditions: to the Rhodium target Xray tube was set to 15kv and 15ma. A vacuum pack was connected to the Spectrometer and a vacuum of 2 torr was pulled. All spectra were collected for 180 seconds. Spectra were taken of the front and the back surface of samples from both marble types treated with each cleaner. This helped to determine if any chemical residue had migrated through the sample.



Figure 12. Jason Church uses the XRF spectrometer to analysis a marble sample after artificial weathering.

There were a variety of results from the first accelerated weathering test. Colorado Yule marble was more likely than Cherokee White marble to display deterioration or discoloration in the accelerated weathering test. NCPTT is currently looking into the

possible reasons that the Colorado Yule marble samples were affected at a greater rate than the Cherokee White marble samples.

There was no discernable change in the samples that were untreated or treated with water. The Colorado Yule marble samples treated with D/2 discolored and took on a slightly translucent appearance. The backs of the Colorado Yule samples also had a very fine powdery deposit on them. When the backside of one sample was examined with the XRF there was a slight Potassium peak. The Colorado Yule samples that were treated with Daybreak discolored to a yellow appearance and had a fine "sandy" coating on the backside. When the backside of one Colorado sample was examined with the XRF a large Chloride peak was detected. The remaining 3 cleaners (Photo-Flo, WEG Marble Cleaner and H2Orange2) had no obvious detectable deterioration.

The thirty three day study represents a worst case scenario where the marble was saturated with a cleaner on a regular base and was not rinsed after cleaning. Any physical change to the marble or chemical deposition on the marble would likely be scene in an extreme situation. Because of the severity represented in the first accelerated weathering study the decision was made to start a second accelerated weathering study.

5.1.1.2. Four Time Cleaning Study

The second accelerated cleaning study used the Q- Panel Lab Products model QUV/ Spray Accelerated Weather Tester preformed at NCPTT began on December 22, 2006 and ended its 800 hour cycle on January 19, 2007. The second accelerated weathering test was an abbreviated version of the first experiment. For the second study only Colorado Yule marble was selected for testing. This decision was made due to the fact that deterioration was evident on the Colorado marble in the first experiment. These samples were prepared from the same marble using the same procedure as in the first accelerated weathering experiment.

For the second experiment only 10 samples were prepared for Weatherometer exposure. These consisted of 2 Colorado Marble replicates for each cleaner. Each sample was sprayed with D/2, Daybreak, WEG Marble Cleaner, or water. Two cleaners, H2 Orange Cleaner and Kodak photo-Flo, were removed excluded from the accelerated weathering, based on results of phase one of the study. Two untreated samples were weathered in this experiment as internal controls. Each of these samples was given a unique 3 digit number that was inscribed on the back of the stone.

One key feature of this study was the decision to only clean the samples weekly, once at the beginning and then three more times at the same cycle on each following Friday. This cleaning schedule may have provided a more realistic approach to the accelerated weathering. Also in this study, the cleaner was sprayed onto the sample then rinsed shortly after being treated according to manufacturers' suggested cleaning directions.

After the 800 hours of weathering was completed, the samples were analyzed in the same steps as the first experiment including laser profilometry and colorimetry. A few additional tests were added to this study to try and get a more detailed view of the stones

reaction to the cleaner. Salt deposits were visible on both the back side of some samples and on the Teflon Weatherometer holder ring that surrounds the stone in place. Gravimetric measurements were taken of each of the stone samples while they were still in the holder. Crystalline grow was visible on the backside of the samples treated with both D/2 and Daybreak.

In addition to the usual photo documentation the samples were also photographed under magnification using both a Leica MZ8 boom microscope at a magnification range of 10x to 50x and a Leica DMRX polarized light microscope at a magnification range of 100x to 500x. Both microscopes were fitted with a Diagnostic Instruments Inc. Digital Spot Camera. Through this process the shape, appearance, growth pattern and relative size of the crystalline growth can be documented.

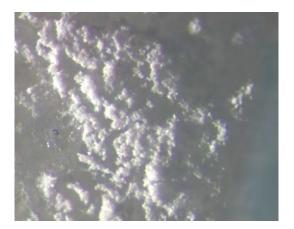




Figure 13. Examples of salt formation on marble samples treated with D/2 (left) and Daybreak (right) both viewed under 100x magnification.

Future research activities will investigate the chemical composition of the efflorescence found on the samples. Both the face and back of all of the samples will be analyzed using the portable XRF Spectrometer. Samples of the salt will be analyzed using X-ray Diffractometry. This will help to determine the bases of the visible salts and any other type of detectable chemical residue left on the stone after cleaning.

Treated and weathered Colorado Yule samples are still being tested and the data processed at this time. New testing methods are currently being considered to maximize the identification of the salt contents in the samples and to identify any other chemical or physical changes that may have taken place in the samples as a result of the cleaner's application.

5.2. Field Studies

Field studies generally consist of evaluating appearance and color changes in the field on field test stones and whole headstones. Additional laboratory analyses of field test stones are described in *Section 4.3*, *Methods of Analysis*, starting on page 38.

5.2.1. Field Stone Samples



Figure 14. NCA staff members Genaro Ocrato and Pat Meyer help set sample stones at San Francisco National Cemetery.

Early in the summer of 2005, 6"x 6" x 24" marble slabs of Colorado Yule and Cherokee White marble were procured from two National Cemetery Administration contracted quarries. These stones would serve NCPTT as the needed field sample stones. Each of the five project cemeteries and the NCPTT labs were shipped a pallet of 22 stones in early June 2005. Of the 22 stones 11 were Colorado Yule and the remaining 11 were Georgia marble. In June, Church visited each of the cemeteries as an initial contact visit. During this visit the sample stones were paired with existing grave markers of the same marble type. Each of the sample stones was set approximately 6" in the ground for stability, see Figure 14.

During the fall trip to each cemetery, ten of the stones were taped into a grid and treated with each of the five cleaners plus water. Of the ten stones half were Colorado and half were Georgia marble. This same process was repeated in the spring on the remaining sample stones, leaving one of each type untouched as a control sample.

In the fall of 2006, each of the stones were removed from the various cemeteries, cling wrapped and stacked on pallets. The pallets have recently been shipped to the laboratories and were received at NCPTT on April 2. They are awaiting testing. The tests preformed on the sample stones will be conducted to look for any physical or chemical changes to the marble itself or to identify any harmful residues left behind by the cleaners that may be harmful over time.

5.3. Methods of Analysis

Staff scientists have determined multiple ways to evaluate accelerated aging samples and field stone samples, based on the criteria established at the beginning of the study by the project team. Important questions to answer include:

- Is the stone appearance changed by the chemical cleaner? If so, is the appearance acceptable?
- Are there physical changes to the stone upon cleaning? Is the stone surface physically altered during the cleaning process with the cleaner? Is the surface rougher or smoother? Is the stone porosity altered during the cleaning process? Are the pores of the stone larger or smaller after cleaning?
- Are there chemical changes to the stone upon cleaning? Does the cleaner interact with the stone to produce chemical changes on the surface? Does the cleaner interact with the stone to produce salts or efflorescence?

5.3.1. Appearance Change

Appearance change is documented using photographic techniques and color measurements. Photographic images are taken of the lab test stones prior to accelerated weathering using the QUV weatherometer. Field test stones were photographed in each cemetery prior to cleaning with the test cleaners. The field tests stones were photographed again after cleaning.

5.3.1.1.Photo-Documentation/Visual Ranking (see section 3.4.1.1.)

NCPTT staff is photographing test samples from laboratory or field test stones under standard lighting on a Kodak gray card using a Polaroid copy stand and color balanced ECT incandescent bulbs. Digital photographs are taken using a Sony DSC-S85 digital camera at 2272 x 1704 pixel resolution. The photographs will be compared visually by at least ten unbiased observers and ranked on the basis of change in appearance.

5.3.1.2.Color Measurement (see section 3.4.1.2.)

Using a Minolta Colorimeter, CR-400, staff made color measurements on lab test samples prior to accelerated aging using the QUV weatherometer. Each measurement was repeated three times on each stone sample and averaged in order to compensate for slight variations in surface texture. The samples were then exposed to UV radiation, temperature cycling, and spray mist cycling as described in section 5.1.1.2. Samples were cleaned weekly for a total for four cleanings. Samples were cleaned either with D2, Daybreak or WEG Marble Cleaner.

Staff will make color measurements of the samples after exposure. The measurements will be taken following the procedures described in the above paragraph. Once the "after' pictures are taken, total color change, ΔE^* , will be calculated for each sample. Samples will be considered unchanged if the ΔE^* is 3 or less. When ΔE^* is 3 or greater, the samples will be considered to have undergone a color change. Shifts in lightness and chroma will be noted.

5.3.2. Physical Change

One aspect of stone deterioration is the change in physical properties of the stone. Physical changes commonly observed in the field include sugaring, blistering, and scaling, among others. Visual observations allow researchers to determine the state of the condition at a moment in time. However, quantification, or putting numbers to the conditions, allows observations over a time range. The NCPTT staff chose two ways to characterize physical changes in the accelerated weathering tests and in the filed test samples. Laser profilometry can be used to quantify the surface of the stone samples. Changes in the pore structure can be measured by porosimetry.

5.3.2.1. Surface Texture

Surface texture is the local variations in the in the surface from its ideal shape. It can be characterized by a number of variables defined by international standards⁸, , including

S_a – Average Roughness for an Area,

 S_p – Highest Peak Surface, the height of the highest peak in the roughness profile over the evaluation area,

 S_v – Valley Depth from surface,

 S_t – The total height of the surface, the sum of $S_{p+} S_{v}$.

 S_{ku} – The Kurtosis, a measure of the randomness of heights and sharpness of a surface,

 S_{vk} – The roughness of the valleys,

 S_k – Roughness of the core

 S_{fd} – the fractal dimension of the surface (complexity of the surface),

 S_q – the root mean square of the roughness, and

 V_v – Void volume of the valleys, among others.

In her doctoral work, ElizaBeth Bede Guin showed that surface texture and porosity can affect the deposition of air pollution on to surfaces. Guin characterized the porosity and surface texture of four different types of high-calcium limestone including Salem, Cordova Cream, Cottonwood Top Ledge, and Monks Park limestones. Half of the stones were chemically etched to create rough surfaces while others were semi-polished to create smooth surfaces. Next the samples were exposed to a simulated polluted sulfur dioxide environment within the NCPTT environmental exposure chamber for 24 hours. The conditions were 50 ppb SO₂; 65% RH; 25°C; 4 m/s wind speed. Deposition velocities were calculated for each stone surface. While porosity was the dominant variable influencing pollution deposition, Guin's work showed that three texture

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⁷ Price, C.A., 1996, *Stone Conservation, an Overview of Current Research*. Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 1-4.

⁸ International Organization for Standards, "Geometrical Product Specifications (GPS) - Surface texture: Profile method; Surfaces having stratified functional properties - Part 2: Height characterization using the linear material ratio curve," ISO 13565-2 1996.

⁹ American Society of Mechanical Engineers, "Surface Texture, Surface Roughness Waviness and Lay," ASME B46.1, 1995.

¹⁰ Bede, ElizaBeth Anne, "The surface morphology of limestone and its effect on sulfur dioxide deposition," Ph.D. dissertation, University of Delaware, 2001, 327 pp.

parameters including S_k , S_{vk} , and S_q , did correlate to the deposition of sulfur dioxide on the stone. ¹¹

Cleaning the surface of the stone may affect both the porosity and the surface texture parameters. The changes may lead to additional soiling by atmospheric pollutants. Alternately, changes may increase moisture retention and lead to increased biological growth.

In this work, we will characterize several surface texture parameters of both laboratory stones and field test stone prior to and after cleaning/accelerated aging or field exposure. Changes in the surface texture from cleaning and/or aging will be noted. Surface texture will be analyzed using laser profilometry on small cut samples.

5.3.2.1.1. Laser Profilometry

Laser profilometery is based on the principle of optical triangulation. It employs a light source (a laser), imaging optics, and a photodetector. The laser is focused on to the surface of the sample. Reflected light is focused on to the photodetector, which generates a signal that is proportional to the position of the spot in its image plane. As the distance to the target surface changes, the imaged spot shifts due to parallax. To generate a three-dimensional image of the stone surface, the sensor is scanned in two dimensions, thus generating a set of distance data that represents the surface topography of the stone ¹².



Figure 15. Researcher scans surface of stone using laser profilometer to determine texture parameters and 3-d profile.

NCPTT uses a Solarius LaserScan, a 3-d non-contact laser profilometer, to characterize stone sample surfaces. The instrument uses a class II diode laser (670 nm wavelength)

¹¹ Bede, Ibid., Chapter 6. (N.B. S parameters reflect area measurements, while R parameters reflect line measurements).

¹² "Introduction to Laser-based Profilometry," Laser Techniques Co., 14508 NE 20th St., Bellevue, WA, 98007 http://www.laser-ndt.com/LP method.pdf (accessed 3/12/2007).

and a 2 μ m spot size. The vertical resolution of this instrument is 0.1 μ m. The maximum vertical range is 1 mm. This range allows for the measurement of surface peaks and valleys typically encountered on stone surfaces. The laser is scanned over an area of 31.07 mm (x-axis) by 23.02 mm (y-axis) at a scan speed of 5 mm/s and a resolution of 25 μ m. ¹³ The estimated run time per sample is 111 minutes.

Laboratory samples chosen for accelerated weathering were documented by laser profilometry using the conditions described in the above paragraph. The samples will be analyzed after exposure and changes in parameters will be calculated.

Field test samples will be measured upon return from the field. This will require cutting small samples from each stone cleaning space. The surface texture will be compared to control samples which have not been exposed in the field, but have been carefully stored in the lab.

5.3.2.2. Stone Porosity

Porosity is the volume of void spaces found in the stone and is expressed as a fraction between 0 -1. The porosity of a stone is important consideration when determining how much water or liquid can be absorbed in a stone or how a stone might be affected by air pollution or long term weathering. Also, a more porous stone may absorb more and retain more cleaner, making it harder to rinse.

Increases in porosity may reflect erosion or material loss from the surface of the stone. This undesirable affect may come from mineral dissolution in water or cleaner. Alternately, decreases in porosity may reflect growth of salts or other residues within the stone pore system.

The voids within a stone have additional characteristics that can be described by size and shape. Large voids in the stone greater than 50 nm are considered macro-pores. Pores in the 50 nm to 2 nm range are considered meso-pores, while pores smaller than 2 nm are called micro-pores. The size of the pores affects the way fluids move through the stone.

5.3.2.2.1. *Mercury Intrusion Porosimetry*

One technique that can determine pore size in a material is called Mercury Intrusion Porosimetry (MIP). It can measure pore size in the range of meso-pores to macropores. Samples are submerged in a confined quantity of mercury and then the pressure of the mercury is hydraulically increased. This forces the mercury into the pores of the material. The results obtained from the instrument include

- pore size distribution (macro/meso range of porosity spectrum),
- hysteresis curve,
- specific surface,
- bulk density,
- total porosity (%), and
- particle size distribution.

¹³ Other conditions include a row pitch of 85.95 and a column pitch of 88.33.

5.3.2.2.2. Nitrogen Absorption Porosimetry

Nitrogen gas absorption can be used to determine the micropores and the lower range of meso-pores in a material. The measurement of adsorption at the gas/solid interface is one of the most widely used techniques for the study of microporous and mesoporous solids. The gas molecule acts as a ruler for the measurement of features at the nanometric scale. Nitrogen is the gas most often used for this type of study. With this technique, a series of isotherms¹⁴ are plotted for the absorption and desorption of nitrogen onto the surface of a stone. ASTM UOP821-81¹⁵ describes a method of determining the distribution of surface area, pore volume (size) and length among the micropores, 60 nm (600 A) and smaller, as well as total surface area, total pore volume and average micropore diameter for porous substances using a Micromeritics Digisorb 2500 Analyzer.

5.3.3. Chemical Change

Criteria for effective cleaners included the ability to provide improved appearance, the efficient removal of biological growth, the deterrence of re-growth, and minimum to trace changes to the physical and chemical nature of the stone. The documentation of appearance change was described in section 4.3.1. Methods used to document physical changes were described in section 4.3.2. This section describes methods used to document chemical changes resulting from cleaning with the test cleaners and is a main focus of phase 2 of the study.

5.3.3.1. Optical Microscopy



Figure 16. Jason Church uses an optical microscope fitted with a digital Spot camera to view salt deposits on the back of a sample

¹⁴ Absorption isotherms are plots of the amount of gas absorbed at equilibrium as a function of the partial pressure at a constact temperature, usually nitrogen at its boiling point.

¹⁵ "UOP821-81 Automated Micro Pore Size Distribution of Porous Substances by Nitrogen Adsorption and/or Desorption Using a Micromeritics Analyzer" ASTM International.

Optical microscopy is a simple but useful analytical method. Microscopy is the ability to view small areas in great detail by using magnification. This technique will be used to view the marble samples that have undergone accelerated weathering. This technique will help determine if there is any visible deposition on the stone. If efflorescence is present due to salt content it will be viewable using optical microscopy. Salt crystals grow in different patterns with varying shapes depending on their composition. The salt crystals shape will be viewable under magnification this will help to determine its composition. The surface of the field stones will be viewed with optical microscopy to check for any visible depositions or crystallization before further testing takes place.

For this analysis NCPTT will make use of its two in-house microscopes; a Leica MZ8 boom microscope with a total magnification range from 6.3X to 50X, and a Leica DMRX polarized light microscope with a magnification range from 50X to 500X. All samples under magnification can be photographed using the microscopes' digital Spot camera attachment. The photographs can provide important visual and comparative documentation.

5.3.3.2. X-ray Fluorescence Analysis

X-ray Fluorescence analysis is a non-destructive method used to determine the elemental composition of a sample. This is done by generating elections using an an X-ray tube. The generated electrons of specific energy bombard the sample. The x-rays can either be absorbed or scattered through the material. The way in which the atom absorbs the x-ray is by transferring the energy to its innermost electron. After this is done the electrons are pushed back from the inner shell causing vacancies. The atom to becomes unstable, and outer shell electrons cascade into the vacancies. This causes the release of energy in the form of X-rays of characteristic energy. Since each element produces x-rays that have a unique energy the elemental composition of the sample can be determined.

This process is accomplished with the use of an XRF Spectrometer and its supporting software. The XRF Spectrometer reads the characteristic energy levels and maps them into a spectrum chart where the elements can be labeled and compared. A comparison of the untreated marble and the marble sprayed with the selected cleaners may show chemical residue left behind due to the cleaners.

A handheld XRF Spectrometer will be used to analysis both marble samples in the laboratory from the accelerated weathering studies as well as the field stone samples that were treated in the cemeteries. Due to the handheld XRF Spectrometer's portability it will also be used to analyze chemical deposition on whole headstones cleaned in the field. NCPTT uses a Tracer III portable X-Ray Fluorescence Spectrometer with a Rhodium target.

5.3.3.3. Scanning Electron Microscopy

Scanning electron microscopy with electron microprobe capabilities permit the observation and characterization of materials.¹⁶ Both techniques are based on irradiating the samples with a finely focused electron beam, which may be swept across the surface

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¹⁶ Goldstein, Joseph I., Dale E. Newbury, Patrick Echlin, David C. Joy, Charles Fiorl, and Eric Lifshin, 1981, *Scanning Electron Microscopy and X-ray Microanalysis*, New York, NY: Plenum Press, Chapter 1.

of a specimen. Different types of signals, including secondary electrons, back-scattered electrons, and characteristic x-rays are produced when the electron beam impinges on the surface of the sample.

The surface topography of a sample can be imaged by collecting secondary and back scattered electrons as the electron beam scans the surface of the sample. This rastered image produces a three dimensional appearance of the surface. Thus, the technique can help elucidate changes in surface texture such as pitting or sugaring.

Additionally, Scanning electron microscopy with energy dispersive spectrometry (EDS) permits the identification of elements present on the surface in major, minor, and trace concentrations. Identification is based on the specific energy of characteristic x-ray peaks for each element and is similar to x-ray fluorescence spectrometry. Also, the surface of the sample can be scanned for these characteristic x-rays, and maps of a specific element can be made on the surface of the sample.

Scanning electron microscopy may be used in this study to provide additional information about possible chemical and physical changes to the field test stones and the artificially weathered stone samples.

5.3.3.4. Total Soluble Salts

While the presence of soluble salts contributes to weathering and decay of porous stone, the decay mechanisms are complex. Soluble salts, such as sodium chloride or calcium sulfate, may damage stone as a result of crystallization pressure or hydration pressure. Crystallization pressure can develop when a supersaturated solution occupies a smaller volume than the precipitating crystals and residual solution. This pressure pushes out on the pores of the stone and causes damage. Alternately, hydration pressure is developed when a salt collects water molecules around itself. Again, the volume needed for hydrated salts is larger than the restrictive pores. Salts push out against the walls of the pores and enlarge the pore space.

Clifford Price points out in his review on stone deterioration that salts represent one of the most important causes of stone decay. Salts may be introduced into stone through rising damp, or blown by the wind. Use of deicing salts can be a problem in colder climates. Unsuitable cleaning may leave salts that ultimately damage the stone.

Based on this knowledge, it is important to determine if any of the cleaning test products leave significant soluble salts on the headstones. Tests to determine total soluble salts in stone include gravimetric and conductivity techniques. NCPTT staff will use one or both methods to evaluate the presence of soluble salts after cleaning in the field and after accelerated studies in the lab.

¹⁷Charola, A. Elena, 2000, "Salts in the Deterioration of Porous Materials: An Overview," *Journal of the American Institute for Conservation*, Vol. 39, No. 3. (Autumn-Winter, 2000), pp 327-343.

¹⁸ Price, C.A., 1996, *Stone Conservation, an Overview of Current Research*. Santa Monica, CA: Getty Conservation Institute, J. Paul Getty Trust, pp 7-9.

5.3.3.4.1. Gravimetric Methods

This test uses weight measurement to determine the soluble salts found in a stone sample. The test method is described in Boyer (1987) as:

A crushed masonry sample of known weight is allowed to interact with distilled water for 24 hours. The sample is then filtered, dried and the precipitate weighed. Water soluble figures are then calculated based on the ratio of weight loss of the precipitate to the original sample. A high water-soluble content would indicate the masonry to be composed of highly water-soluble materials which would reduce its resistance to weathering.¹⁹

5.3.3.4.2. Electrical Conductivity Method

A second method that can be used to investigate salts and other soluble contents within the stone is the use of electrical conductivity measurements. Electrical conductivity is directly related to the concentration of dissolved ionized solids in a wash solution. Again, the sample is ground, then soaked in distilled water for 24 hours. The solution is filtered through a filter paper of 2 micrometer pores. Then an electrical conductivity meter is used to measure the conductivity of the solution in micro-siemens. Higher electrical conductivities indicate greater total dissolved solids.

6. Comments and Discussion

6.1. Appearance

Appearance changes of field trails were documented using photography and colorimetry throughout phase one of the study. Very subtle changes were seen on stones over time from six months to twelve months after cleaning. However these changes were often not noticeable to the viewer. None of the cleaners left obvious changes, such as yellowing, etc., from possible cleaning residues.

Color change trends were examined by determining the frequency of color changes at ΔE greater than 5 and ΔE greater than 10. Trends were evaluated by cemetery, by cleaners, and by sunny or shady locations. In most cases where color change occurred, headstones were darkening.

From frequency trend data associated with cleaners, Kodak Photo-Flo exhibited the greatest number of color changes greater than 5 ΔE and greater than 10 ΔE , and was likely the worst performer of the test cleaners. None of the other cleaners were readily distinguished based on changes in visual appearance.

It is important to note that, while H₂Orange cleaner seemed to perform well based on color measurements, significant visual changes were noted over a six month time period. The appearance of biological re-growth or staining was not always captured by color measurements, since changes often occurred at the outer edges of the headstone.

¹⁹ David W. Boyer, 1987, "A Field and Laboratory Testing Program: Determining the Suitability of Deteriorated Masonries for Chemical Consolidation," *APT Bulletin*, Vol. 19, No. 4, 1987, pp. 45-52.

Moreover, after twelve months, the visual changes had disappeared. Despite the fact that this phenomena was observed at only one cemetery, Jefferson Barracks National Cemetery, it was deemed to be an unacceptable short term appearance change.

Appearance changes were subtle during the six and twelve month time period. In general, more time is needed to see significant appearance changes to the headstones.

6.2. Biological Re-growth

Determination of biological re-growth in this study has offered some complex problems, from the sheer numbers of samples to be evaluated and enumerated, to how cleaning history of the stones affect the initial biological activity, to the length of time needed for observing visual biological re-growth.

Biological swabs were taken from many headstones and required considerable time and effort to enumerate in the course of this study. Initial estimates of the number of samples to be examined were 7,880 biological counts, taking over 63,000 hours of work to perform! This was an impossible task and in June 2005, we revised the number of samples to 600 swabs. Still the task was daunting and ultimately, fewer samples were evaluated.

All headstones started with a relatively small biofilm of bacteria and fungi at the beginning of the study, with the exception of headstones located in Santa Fe National Cemetery which displayed a larger biofilm. This is likely due to the fact that Santa Fe headstones are not regularly cleaned in the same manner as those located in the other test cemeteries. Importantly, no algaes or photosynthetic bacteria were observed in the samples. According to Dr. Ralph Mitchell,²⁰ it is likely that algaes or photosynthetic bacteria are the greatest source of visual appearance change found on headstones and thus are the most important to enumerate. Fungi are also sources of visual discoloration, but to a lesser extent.

As of November and December 2006, no algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.

NCPTT staff attempted to identify performance trends based on the biological activity documented over the course of twelve months. Performance of each cleaner was ranked based on data from swabs. Rankings from June 2006 results appeared to illuminate differences to a greater extent than rankings from February 2007. This is partly due to

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²⁰ Mitchell, Ralph. Harvard University, Division of Engineering and Applied Sciences, personal communication, March 2007.

the fact that there were six cleaners to rank in June 2006 where as there were four cleaners to rank in February 2007. The latter rankings grouped more closely together thus making it more difficult to see significant differences.

Based on the June 2006 rankings, Kodak Photo-Flo was likely the worst performer of the six cleaners evaluated.

6.3. Physical Changes

Evaluation of physical changes is a significant task in phase 2 of the study. Physical changes will be evaluated for field test stones and for accelerated weathering laboratory samples. To date, NCPTT staff has identified methods to be used in evaluating physical changes to the stones. They include changes in appearance by colorimetry, changes in surface texture to be monitored by laser profilometry, and changes in porosity to be examined by mercury porosimetry and Nitrogen BET absorption porosimetry.

Laboratory samples were examined using colorimetry, laser profilometry, and weight measurements prior to any accelerated weathering studies as the baseline data. Field test stones will be compared to control samples kept in pristine conditions in the laboratory.

This work is on-going.

6.4. Chemical Changes

As with the evaluation of physical changes, chemical changes caused by cleaners will be evaluated in the laboratory as part of phase 2 of the study. The possible presence of soluble salts will be evaluated using optical microscopy and analysis of total soluble salts using both gravimetric and conductivity methods. The chemical nature of the efflorescence may be studied using X-ray Diffraction analysis. Detection of possible cleaning residues or minor chemical changes may be studied using Electron Microscopy-EDS, and X-ray Fluorescence Spectroscopy.

NCPTT staff has tested its new portable XRF analyzer for identifying chlorides on field test stones with success. The task of identifying chemical changes to the stones continues.

6.5. Issues Associated with Bath National Cemetery



Figure 17. Signage in Section F of Bath National Cemetery.

On November 7, 2006 Church arrived at the Bath National Cemetery to begin work on the first part of phase two of the project. The first noticeable thing in the cemetery was that sections A, B, D and F had recently gone through a section renovation which consists of the raising and realignment of each of the headstones in the section. This affected headstones involved in the project in several ways, including soiling, contamination and total loss of the stones themselves.

Section F is included in the test study as the "Sunny Section." There are 24 Headstones and 22 lab sample stones located in section F. Of these headstones and field test stones, all were affected by general soiling. This was caused when the headstone was dug out of the ground and laid aside during resetting. Ongoing monitoring of the visual appearances of stones have been altered by this soiling, see Figure 18. Also, in the renovation process two of the lab sample stones were moved and reset one row up from where they had been placed, thus losing their connection to the original headstone.



Figure 18. Headstone F712 in April 2006 and again after resetting in November 2006.

Through out the sections A, B, D and F, hydro-seeding was used to help with erosion and to replace the ground cover that was lost during renovations. Hydro-seeding is the process of planting mass quantities of grass seed by spraying a slurred mixture over a large area. The mixture for hydroseeding contains five basic components including – a recycled paper pulp, grass seed, tracking dye, fertilizer and a tackifier. The tackifier usually is a linear polyacrylamide polymer that electrochemically binds soil particles. This process could have contaminated the biological data as well as the colorimeter and visual inspection of the all the headstones involved.



Figure 19. Headstone F812, note that the tan spots are clumps of hydro-seed still attached to the marble.

The effects of the hydro-seeding are visible in section F where the headstones are directly beside the lab sample stones. In the hydro-seeding process the cemetery maintenance staff placed small plastic bags over the headstones to shield them from the grass slurry. This process was not photographed at the time the work was being done. This seams to have been adequate protection for most of the grave markers, with the exception of any headstone that was set beside a lab stone. These headstones and lab stones still retained a spotty coating of the hydro-seed.

Sections B and D on the cemetery are mostly Spanish American War markers. Church was told by Bath's maintenance staff that most of these were originally set with concrete around the base of the stone. During the renovation of sections B and D several stones were broken in attempts to raise and realign them. All historic stones broken in this section were replaced with newly carved Georgia marble that mimic the original markers' design and font. Four of the stones broken and replaced in this section were in the cleaning study. They were

- D 7 14 Albert McKinzie
- B 1 8 SE Catlin
- B 1 9 Peter Welch
- B 1 10 Adam Graf

Biological and colorimeter data had been collected on these stones. When asked about the stones the staff stated that they were unaware of which stones had been replaced and that it was believed only the lab samples and their corresponding headstones were still in the study.





Figure 20. Marker B18 in April 2006 and its replacement in November 2006.

7. Recommendations

The following recommendations are based on data taken in the field from June 2005 to March 2007, from analysis of biological activity performed at the Laboratory of Applied Microbiology at Harvard University, and NCPTT staff research experiences during the course of this study. There are three main recommendations – the elimination of two cleaners, the elimination of Bath National Cemetery from the study, and the continuation of the study for an additional time period.

7.1. Elimination of Cleaners

- Kodak Photo-Flo was eliminated from the first part of phase one based on the performance rankings of the biological activity seen in June 2006.
- Kodak Photo-Flo was likely the worst performer based on frequency of color change data as well.
- H₂Orange cleaner was eliminated from the first part of phase one based on visual examination of headstone test patches at Jefferson Barracks National Cemetery, as observed six months after cleaning.
- Three cleaners continue to be studied, including D/2 Antimicrobial cleaner, Daybreak, and WEG Marble cleaner.

7.2. Bath National Cemetery

- Unique problems are associated with phase one of the study at Bath National Cemetery as documented in section 5.5 of this report.
- It is unlikely that data from the cleaning test patches or field test stones will provide meaningful data, since they were likely contaminated during the recent

- section renovation. Moreover, four headstones were broken and completely lost during the renovation.
- NCPTT staff recommends that the NCA consider eliminating the evaluation of whole headstones at Bath National Cemetery.

7.3. Continuation of Study

- NCPTT staff recommends extending the study for an additional time frame of two vears.
- Subtle appearance changes, the variability of biological growth, and the absence of algae support this recommendation.
- NCPTT offers four options for consideration:
 - Option A is to continue the study at four cemeteries, including field trips annually, with additional funding for travel, salary, and evaluation of biological activity.
 - Option B is to continue the study at two cemeteries, ²¹ including field trips annually, with additional funding for travel, salary, and evaluation of biological activity.
 - Option C is to continue the study at Alexandria National Cemetery, including field trips every six months, with additional funding for salary, and evaluation of biological activity annually.
 - Option D is to continue the study at Alexandria National Cemetery, including field trips every six months, with minimal additional funding for salary only. Appearance changes would be examined; no biological testing would be planned.
- Cost considerations for the above recommendations are found in Appendix I.

8. Appendices

Appendix A. Photographic Documentation of Field Trials
Appendix B. Color Measurements on Field Trials
Appendix C. Color Analyses by Cemetery, Test Patch, and Location

Appendix D. Analysis of Microorganisms on headstones in VA Cemeteries,

First Report: December 2005

Appendix E. Analysis of Microorganisms on Headstones in VA Cemeteries,

Second Report: June 2006

Appendix F. Analysis of Microorganisms on Headstones in VA Cemeteries,

Third Report: February 2007

Appendix G. Biological Performance Based on June 2006 Report
Appendix H. Biological Performance Based on February 2007 Report

Appendix I. Cost Estimates, Four Options for Continuing the Study for Two Years

²¹ We recommend continuation of the study at Jefferson Barracks National Cemetery and Alexandria National Cemetery. We would eliminate San Francisco National Cemetery because cleaning maintenance is contracted out and Santa Fe National Cemetery may not show significant visual appearance change because of the hot dry climate.

Appendix A. Photographic Documentation of Field Trials

October 4, 2005 B 1200-A

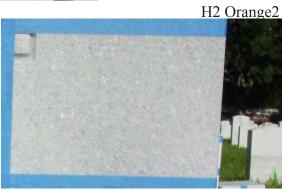




Daybreak







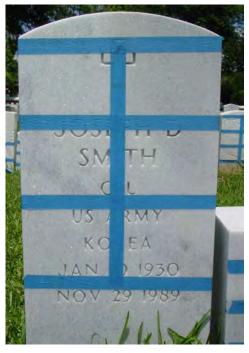
Marble Cleaner Conc.



Photo-flo



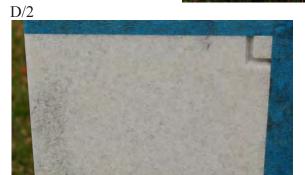
May 17, 2006 B 1200-A





January 16, 2007 B 1200-A





Daybreak



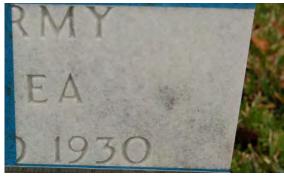




Marble Cleaner Conc.



Photo-flo



October 4, 2005 B 1312









Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



May 17, 2006 B 1312















January 16, 2007 B 1312









Water





Marble Cleaner Conc.



Photo-flo



October 4, 2005 C 418-A





May 17, 2006 C 418-A





January 16, 2007 C 418-A





Daybreak







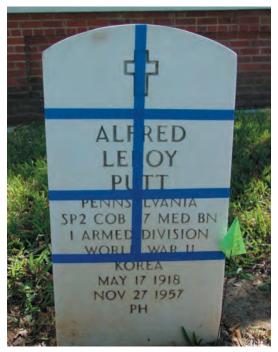
Marble Cleaner Conc.



Photo-flo



October 4, 2005 C 417-A





Daybreak







Marble Cleaner Conc.



Photo-flo

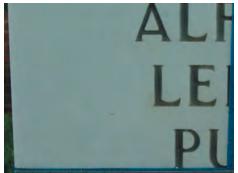


May 17, 2006 C 417-A

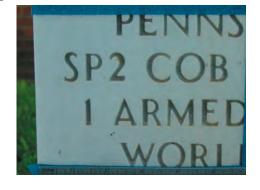








Water





Marble Cleaner Conc.



Photo-flo



January 16, 2007 C 417-A



D/2 H2 Orange2



Daybreak



Water





Marble Cleaner Conc.



Photo-flo



May 17, 2006 B 1201-A





January 16, 2007 B 1201-A





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



May 17, 2006 B 1202











H2 Orange2

Marble Cleaner Conc.



Photo-flo



January 16, 2007 B 1202





Daybreak







Marble Cleaner Conc.



Photo-flo



May 17, 2006 C 419





Daybreak



CPL CO 39 DI WORL



Marble Cleaner Conc.



Photo-flo



January 16, 2007 C 419





Daybreak







Marble Cleaner Conc.



Photo-flo



May 17, 2006 K 151





January 16, 2007 K 151





Daybreak







Marble Cleaner Conc.



Photo-flo



October 6, 2005 A2 1-B





April 4, 2006 A2 1-B





November 7, 2006 A2 1-B

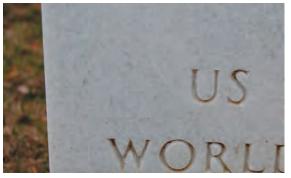




Daybreak



Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



October 6, 2005 D 713





Daybreak







Marble Cleaner Conc.



Photo-flo



April 4, 2006 D 713





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



November 7, 2006 D 713





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



October 6, 2005 F 215





April 4, 2006 F 215





Daybreak







Marble Cleaner Conc.



Photo-flo



November 7, 2006 F 215



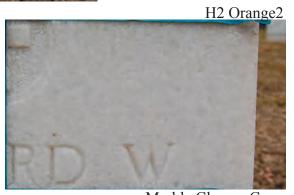






Water





Marble Cleaner Conc.

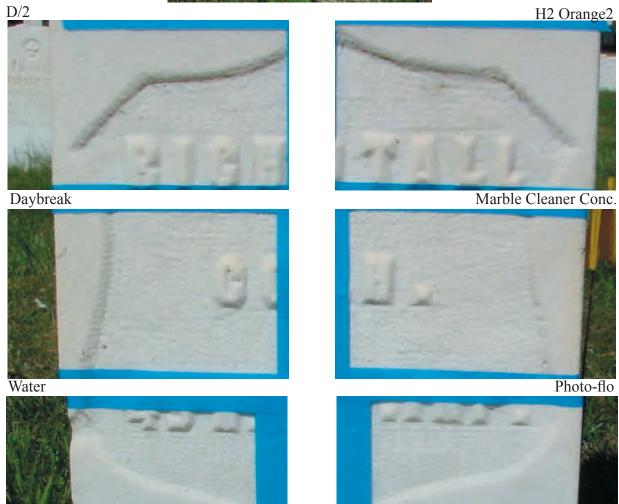


Photo-flo



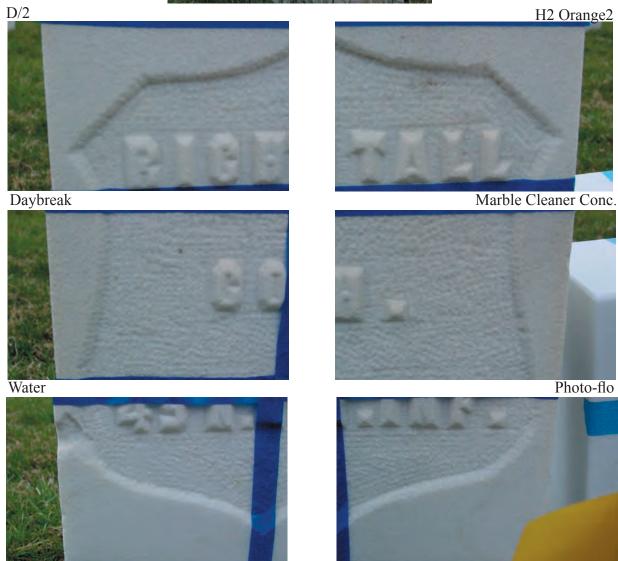
October 6, 2005 F 712





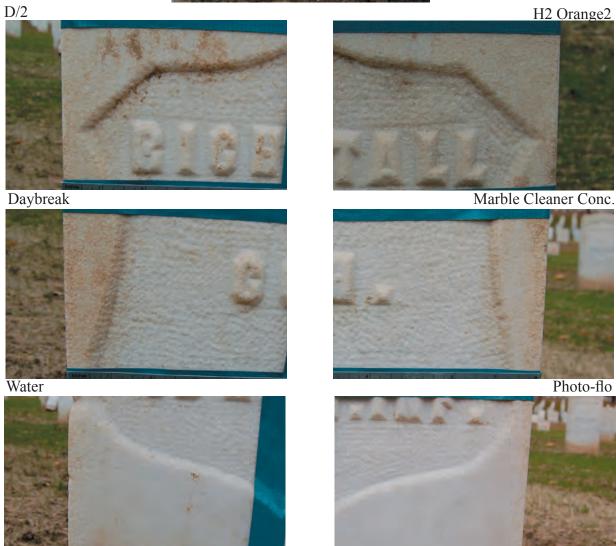
April 4, 2006 F 712





November 7, 2006 F 712





April 4, 2006 A 132





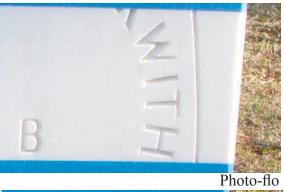
Daybreak



Water









November 7, 2006 A 132









Water



H2 Orange2

Marble Cleaner Conc.

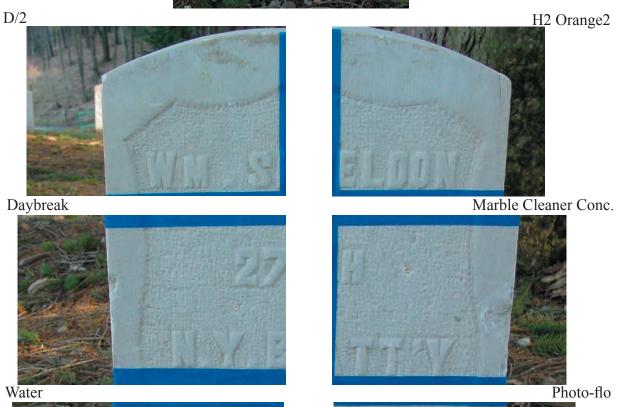


Photo-flo



April 4, 2006 B 112





November 7, 2006 B 112





Daybreak



Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



April 4, 2006 F 513





November 7, 2006 F 513





Daybreak



Water





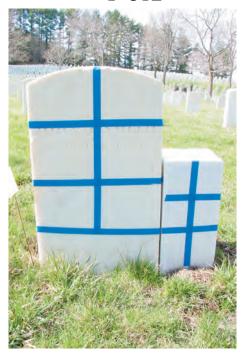
Marble Cleaner Conc.

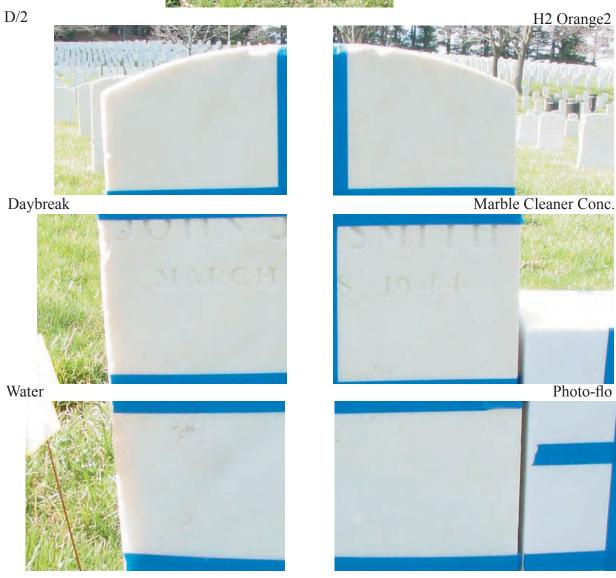


Photo-flo



April 4, 2006 F 812





November 7, 2006 F 812









Water





Marble Cleaner Conc.

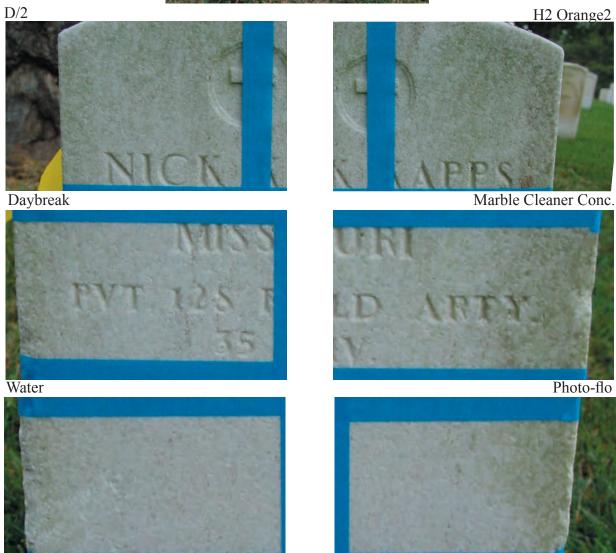


Photo-flo



October 19, 2005 32 2898-A





April 11, 2006 32 2898-A









Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



April 11, 2006 32 2898-A









Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



October 19, 2005 32 2904-A





Daybreak



Water





Marble Cleaner Conc.



Photo-flo

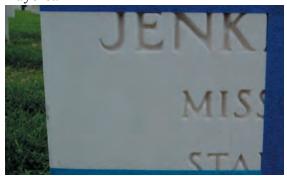


April 11, 2006 32 2904-A





Daybreak



Water





Marble Cleaner Conc.





November 9, 2006 32 2904-A





Daybreak



Water





Marble Cleaner Conc.

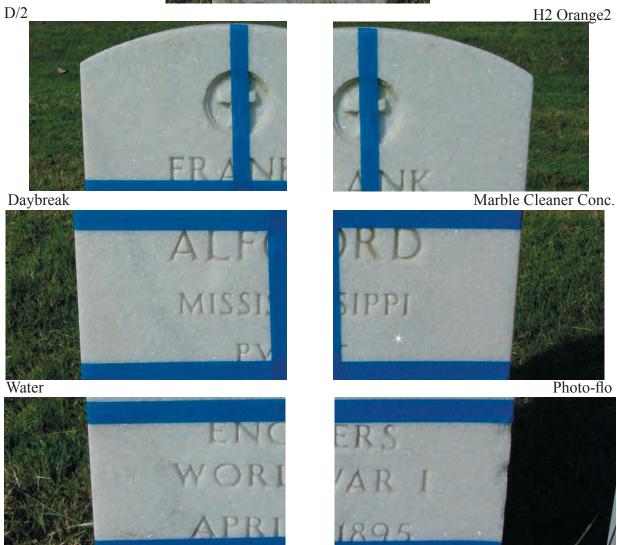


Photo-flo



October 19, 2005 72 1273





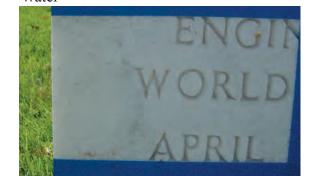
April 11, 2006 72 1273













Marble Cleaner Conc.



Photo-flo



November 9, 2006 72 1273



D/2





Water





Marble Cleaner Conc.



Photo-flo



October 19, 2005 72 1370

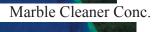












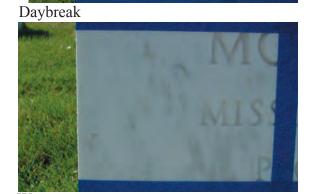




April 11, 2006 72 1370















November 9, 2006 72 1370















April 11, 2006 3151





November 9, 2006

3151





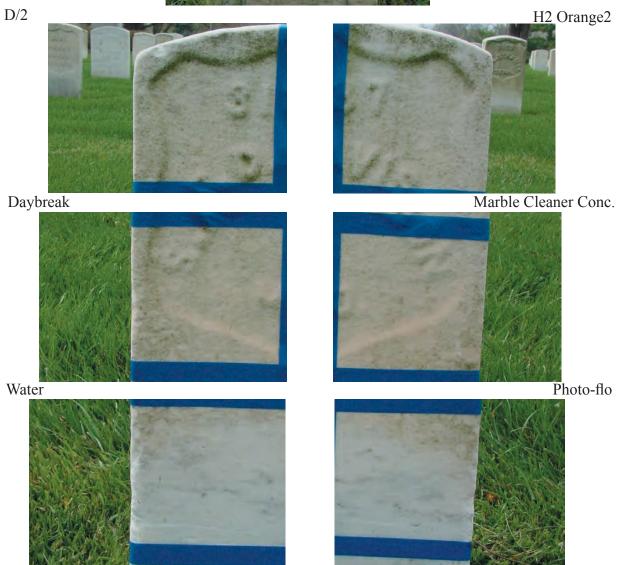




H2 Orange2

April 11, 2006 3187





November 9, 2006 3187















April 11, 2006 72 1164





November 9, 2006 72 1164













Marble Cleaner Conc.



Photo-flo



April 11, 2006 72 1268















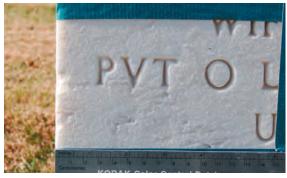
November 9, 2006 72 1268







Water



Marble Cleaner Conc.

H2 Orange2



Photo-flo



November 2, 2005 NAWS 881B



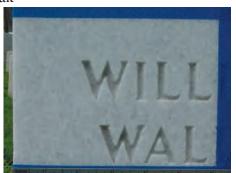


April 26, 2006 NAWS 881B









Water





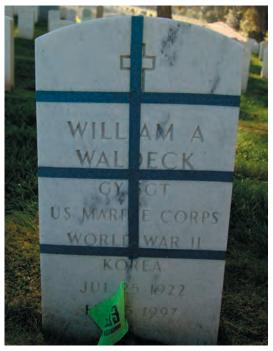
Marble Cleaner Conc.



Photo-flo



December 5, 2006 NAWS 881B

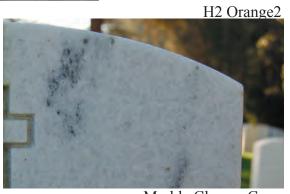




Daybreak







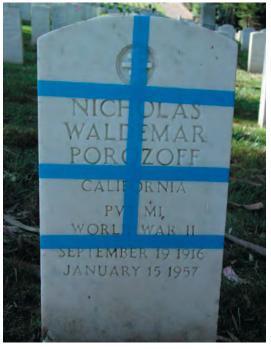
Marble Cleaner Conc.



Photo-flo



November 2, 2005 NAWS 886B









Water





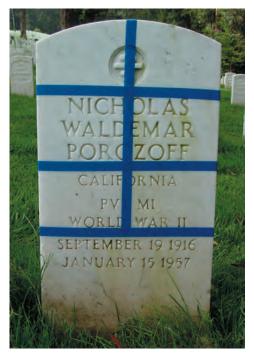
Marble Cleaner Conc.



Photo-flo

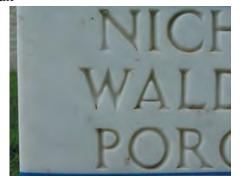


April 26, 2006 NAWS 886 B





Daybreak



Water





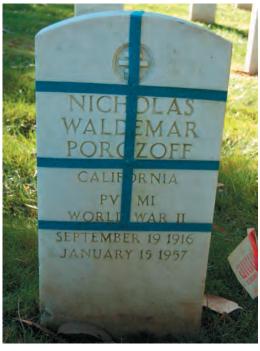
Marble Cleaner Conc.



Photo-flo



December 5, 2006 **NAWS 886B**











Marble Cleaner Conc.





November 2, 2005 WS 1032B





April 26, 2006 WS 1032B















December 5, 2006 WS 1032B









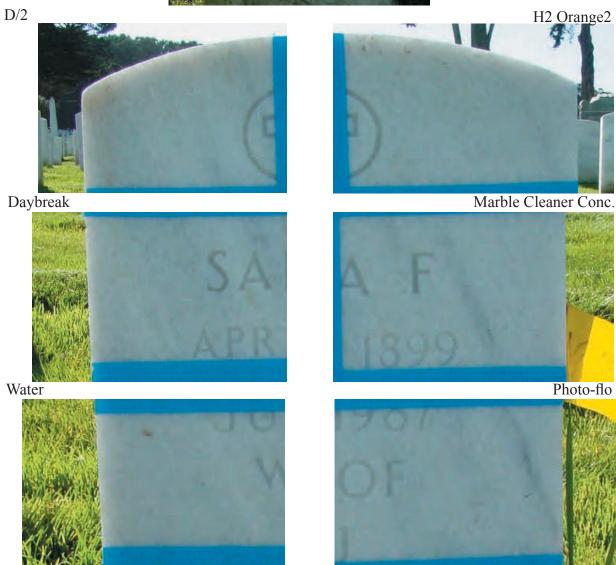






November 2, 2005 WS 1033B





April 26, 2006 WS 1033B





December 5, 2006 WS 1033B





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



April 26, 2006 1075





December 5, 2006 1075















April 26, 2006 NAWS 739B





December 5, 2006 NAWS 739B















April 26, 2006 WS 862B





December 5, 2006 WS 862 B







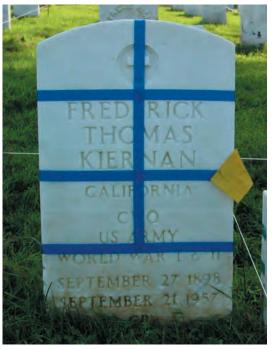








April 26, 2006 WS 1038B





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



December 5, 2006 WS 1038B













Marble Cleaner Conc.



Photo-flo



Santa Fe National Cemetery

November 11, 2005 H 526 D









Water





Marble Cleaner Conc.



Photo-flo



Santa Fe National Cemetery

May 3, 2006 H 526 D









Water



H2 Orange2

Marble Cleaner Conc.



Photo-flo



Santa Fe National Cemetery

December 7, 2006 H 526 D



D/2 H2 Orange2



Daybreak



Water





Marble Cleaner Conc.



Photo-flo



November 11, 2005 H 526 J





May 3, 2006 H 526 J





Daybreak







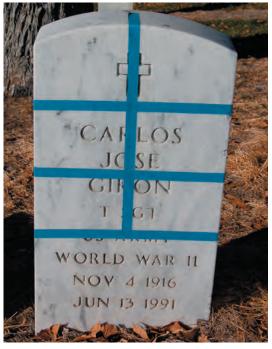
Marble Cleaner Conc.



Photo-flo



December 7, 2006 H 526 J





Daybreak







Marble Cleaner Conc.



Photo-flo



November 11, 2005 U 311-A













Marble Cleaner Conc.



Photo-flo



May 3, 2006 U 311-A









Water





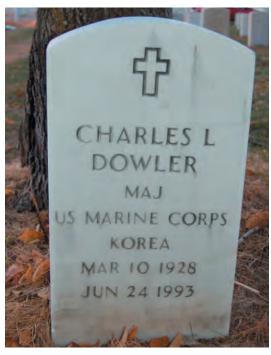
Marble Cleaner Conc.



Photo-flo



December 7, 2006 U 311-A













Marble Cleaner Conc.



Photo-flo



November 11, 2005 U 343









Water



H2 Orange2

Marble Cleaner Conc.





May 3, 2006 U 343





December 7, 2006 U 343









Water



H2 Orange2

Marble Cleaner Conc.

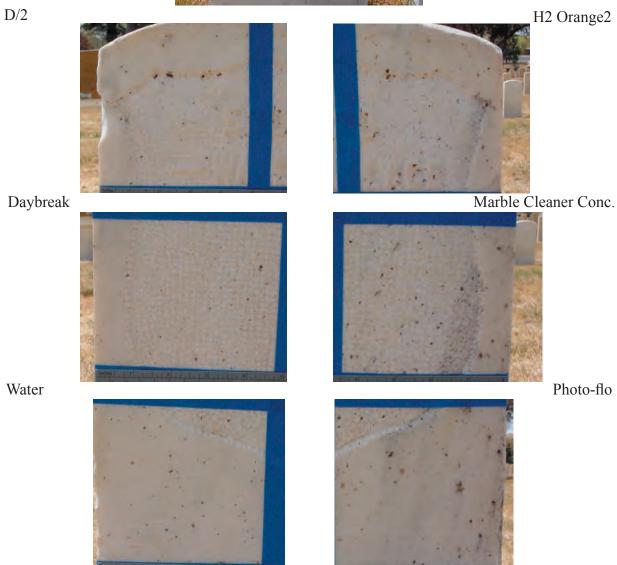


Photo-flo



May 3, 2006 H 530





December 7, 2006 H 530





Daybreak







Marble Cleaner Conc.



Photo-flo



May 3, 2006 I 444





December 7, 2006 I 444





Daybreak







Marble Cleaner Conc.

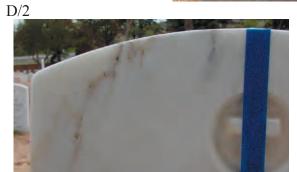


Photo-flo



May 3, 2006 U 280





Daybreak









Marble Cleaner Conc.

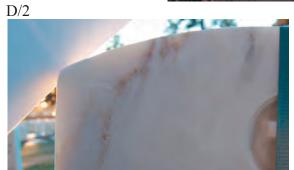


Photo-flo



December 7, 2006 U 280





Daybreak



Water





Marble Cleaner Conc.



Photo-flo



May 3, 2006 U 311















December 7, 2006 U 311









Water



Marble Cleaner Conc.

H2 Orange2



Photo-flo



Appendix B. Color Measurements on Field Trials

Alexandria I	NC
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Alexandi	4-Oct-05				17-May-06				16- Jan-07	16-Jan-07			May 17 2006	to Januar				
Grave		Average L* Av	/erage a* Av	erage b*		B 1202 Average L* Average a* Average b*				Average L* A	verage a* Av	verage h*		May 17, 2006 to January 16, 2007 Δ L* Δ a* Δ b* Δ E				
Space	1	/ wordgo E / w	rorago a rii	orago b	1	81.73	-0.05	2.62	B 1202		-0.08	1.80	0.76	-0.03	-0.82	1.12		
Орасс	2				2		-0.13	1.47	2		-0.49	1.89	-5.02	-0.36	0.42	5.05		
	3				3		-0.52	2.38	3		-0.43	1.04	-2.89	0.09	-1.34	3.19		
	4				4	82.20	0.00	2.83	2		-0.02	2.64	-0.03	-0.02	-0.19	0.19		
	5				5	76.77	-0.10	1.52	5		-0.62	2.35	-6.53	-0.52	0.83	6.60		
	6				6		-0.51	1.79	ě		-0.58	1.60	-3.87	-0.07	-0.19	3.88		
	1312		0.15	4.54	1312 1		-0.46	6.59	1312		-0.66	7.61	1.61	-0.20	1.02	1.92		
	1				2													
	2		0.23 0.32	5.56	3		-0.51	6.94	2		-0.70	7.19	0.62	-0.19	0.25 -0.82	0.69		
	4			7.55	3		-0.49	5.94	2		-0.70	5.12	-1.90	-0.21		2.08		
		66.39	0.42	6.16			-0.37	6.79			-0.82	8.65	-0.19	-0.45	1.86	1.92		
	5 6		0.20 0.33	6.69 6.78	5 6		-0.39 -0.40	8.34 5.41	5		-0.63 -0.71	6.91 7.36	2.88 -4.13	-0.24 -0.31	-1.43 1.95	3.22 4.58		
	B 1200 A	71.57	0.21	3.29	B 1200 A	74.43	-0.04	0.75	B 1200 A	74.39	-0.14	0.79	-0.04	-0.10	0.04	0.11		
	2		0.25	2.74	2		0.03	0.19	2		-0.08	0.73	0.66	-0.10	0.04	0.67		
	3		0.25	3.90	3		0.03	-0.44	3		-0.12	-0.32	-1.68	-0.11	0.12	1.69		
	4	61.99	0.30	4.91	4		0.01	1.38	2		-0.12	1.18	3.73	-0.13	-0.20	3.74		
	5		0.24	3.19	5		0.09	0.86			-0.02	0.54	3.91	-0.11	-0.20	3.93		
	6		0.27	3.18	6		-0.09	-0.07	6		-0.12	-0.01	2.10	-0.22	0.06	2.11		
	K 151				K 151	00.04	0.45	0.40	K 151	00.70	0.04	4.05	0.44	0.40	0.04	0.04		
	1				1		-0.15	2.16	1		-0.34	1.25	0.11	-0.19	-0.91	0.94		
	2				2		-0.16	0.57	2		-0.38	0.49	-0.69	-0.22	-0.08	0.73		
	3				3		-0.12	0.25	3		-0.59	2.25	2.55	-0.47	2.00	3.27		
	4				4		-0.10	1.48	4		-0.31	1.38	-3.09	-0.21	-0.10	3.10		
	5 6				5 6		-0.18 -0.11	0.96 1.17	5		-0.26 -0.34	-0.11 1.37	-9.43 0.69	-0.08 -0.23	-1.07 0.20	9.49 0.75		
	0				0	07.51	-0.11	1.17	,	00.20	-0.34	1.57	0.09	-0.23	0.20	0.75		
	C 419				C 419				C 419									
	1				1		-4.01	12.32	1		-0.55	2.87	9.08	3.46	-9.45	13.55		
	2				2		-1.50	6.44	2		-0.71	2.05	-6.78	0.79	-4.39	8.12		
	3				3		-0.87	5.30	3		-0.78	2.48	-3.55	0.09	-2.82	4.53		
	4				4		-3.99	13.50	4		-0.36	3.31	7.08	3.63	-10.19	12.93		
	5				5		-1.26	7.97	5		-0.65	3.17	-6.34	0.61	-4.80	7.98		
	6				6	73.89	-0.74	5.75	6	70.09	-0.84	3.59	-3.80	-0.10	-2.16	4.37		
	B 1201 A				B 1201 A				B 1201 A									
	1				1		-0.02	-2.32	1		-0.17	-1.09	-1.50	-0.15	1.23	1.95		
	2				2		0.07	-1.99	2		-0.21	-1.86	-6.07	-0.28	0.13	6.08		
	3				3		0.06	-2.06	3		-0.25	-0.80	1.39	-0.31	1.26	1.90		
	4				4		-0.01	-2.39	4		-0.33	0.09	5.22	-0.32	2.48	5.79		
	5				5	71.64	0.03	-2.39	5		-0.28	-1.91	-1.29	-0.31	0.48	1.41		
	6				6	70.83	-0.01	-2.30	6	71.32	-0.26	-0.86	0.49	-0.25	1.44	1.54		
	C 417-A				C 417-A				C 417-A									
	1	84.02	0.02	2.01	1		-0.03	1.76	1		-0.38	1.75	-8.37	-0.35	-0.01	8.38		
	2		0.00	2.03	2		-0.14	1.98	2		-0.53	3.00	-6.44	-0.39	1.02	6.53		
	3	83.50	0.04	2.08	3	75.90	-0.25	3.86	3	70.69	-0.64	7.28	-5.21	-0.39	3.42	6.24		
	4		0.04	2.08	4	81.53	-0.05	1.94	4		-0.41	2.54	-6.17	-0.36	0.60	6.21		
	5		0.05	1.98	5	79.26	-0.07	1.79	5		-0.50	3.95	-6.00	-0.43	2.16	6.39		
	6	83.21	0.03	2.02	6	75.68	-0.24	5.14	6	71.25	-0.69	8.19	-4.43	-0.45	3.05	5.40		
	C 418-A				C 418-A				C 418-A									
	1	68.32	0.11	2.63	1	66.70	0.02	1.12	1	69.53	-0.15	1.25	2.83	-0.17	0.13	2.84		
	2	69.78	0.16	3.12	2	68.43	-0.10	1.21	2	66.22	-0.38	2.03	-2.21	-0.28	0.82	2.37		
	3	68.25	0.02	2.16	3	66.08	-0.12	1.98	3	67.17	-0.41	3.12	1.09	-0.29	1.14	1.60		
	4	65.62	-0.03	0.75	4	68.60	0.01	1.27	4	68.57	-0.17	1.09	-0.03	-0.18	-0.18	0.26		
	5	69.17	0.04	0.81	5	68.50	0.02	1.17	5	67.12	-0.31	1.86	-1.38	-0.33	0.69	1.58		
	6	67.07	0.01	1.59	6	68.26	-0.09	2.57	6	65.25	-0.46	3.60	-3.01	-0.37	1.03	3.20		

Jefferson Barracks National Cemetery Difference from 4/1/2006 11/1/2006 4/1/06 to 11/1/06 10/1/2005 32 2904-A Average L* Average a* Average b* 32 2904-A Average L* Average a* Average b* ΔΕ Grave 32 2904-A Average L* Average a* Average b* Δa* Δb* -0.54 0.29 3 25 Space 81 26 0.22 5.34 82.80 0.52 5.23 85 99 -0.02 5.52 3.19 -0.43 0.95 1.09 79.04 0.24 5.11 84.05 0.41 5.78 83.72 -0.02 6.73 -0.33 80.97 0.55 5 99 81 84 0.12 4 33 78.70 -0.23 5.70 -3 14 -0.35 1.37 3 44 3 3 3 80.28 0.22 5.49 72.26 -0.56 8.55 4 74.79 0.04 6.37 2.53 0.60 -2.18 3.39 5 80.86 0.13 5.46 5 81.05 0.22 7.08 5 83.43 -0.09 6.75 2.38 -0.31 -0.33 2.42 82.63 0.52 5.04 82.67 0.24 5.29 77.61 0.01 6.29 -5.06 -0.23 1.00 5.16 32 2898-A 32 2898-A 32 2898-A 71.52 4.37 71.68 0.62 4.90 72.37 0.19 5.99 0.69 -0.43 1.09 1.36 0.57 2 70.22 0.48 5.12 2 75.22 0.43 4.95 75.88 0.11 5.42 0.66 -0.32 0.47 0.87 3 71.41 0.50 4.62 75.18 0.42 6.05 3 74.54 0.19 7.32 -0.64 -0.23 1.27 1.44 3 4 73.69 0.65 4.63 4 57.47 -1.84 9.13 4 72.12 -0.08 5.03 14.65 1.76 -4.10 15.31 68.76 0.98 5.86 71.35 -0.28 6.91 75.25 -0.07 6.13 3.90 0.21 -0.78 3.98 5 5 5 6 70.10 0.63 5.08 74.78 0.25 6.21 6 74.85 -0.03 7.24 0.07 -0.28 1.03 1.07 3151 3151 3151 57.70 -1.68 9.55 75.46 -0.13 4.69 17.76 1.55 -4.86 18.48 1 2 2 74.16 0.26 6.29 2 59.21 0.08 5.15 -14.95 -0.18 -1.14 14.99 3 3 70.88 0.16 4.05 3 73.26 -0.13 3.12 2.38 -0.29 -0.93 2.57 54.48 9.25 20.35 1.88 -2.27 20.56 4 4 -1.71 4 74.83 0.17 6.98 75.54 0.23 7.74 78.16 -0.13 7.59 2.62 -0.36 -0.15 2.65 6 70.38 0.00 4 33 6 75.86 -0 14 4 27 5.48 -0 14 -0.06 5.48 3187 3187 3187 69.29 0.41 11.12 78.25 0.16 9.74 8.96 -0.25 -1.38 9.07 2 72.06 0.84 13.64 2 68.48 1.41 13.23 -3.58 0.57 -0.41 3.65 2 3 78.33 0.15 7.77 3 75.25 -0.59 2.39 -3.08 -0.74 -5.38 6.24 70.63 -0.20 10.72 75 61 0.08 8 68 4.98 0.28 -2.04 5.39 4 73.43 0.98 13.64 72.40 1.51 13.90 -1.03 0.53 0.26 1.19 6 76.44 0.07 9.66 73.52 -0.60 2.41 -2.92 -0.67 -7.25 7.84 72 1273 72 1273 72 1273 70.86 8 53 77 97 0.30 5.59 75.15 0.12 6 23 -2 82 -0.18 0.64 2.90 1.28 1.71 9.65 5.60 75.21 5.13 -3.75 -0.28 -0.47 3.79 2 70.23 2 78.96 0.22 2 -0.06 1.32 0.12 5 43 71.14 -0.13 -7 75 7.83 3 71 92 8.81 3 78 89 3 4 32 -0.25-1.11 71.31 1.05 73.35 -2.56 6.82 75.91 0.35 5.53 -0.02 5.62 -0.37 0.09 2.59 0.22 -0.34 6.50 -12.91 12.92 5 72.12 0.90 6.76 77 28 6.51 5 64 37 -0.56 -0.01 6 71.76 0.96 6.72 77.40 0.22 5.81 6 68.46 0.33 6.64 -8.94 0.11 0.83 8.98 72 1164 72 1164 72 1164 75.65 7.05 57.72 5.31 -17.93 -0.15 -1.74 18.01 0.49 0.34 2 75.31 0.49 6.68 2 64.02 0.38 5.10 -11.29 -0.11 -1.58 11.40 0.39 63.13 0.17 -9.50 -0.94 3 72.63 4.91 3 3.97 -0.229.55 74.80 0.46 5.70 4 64.08 0.44 5.23 -10.72 -0.02 -0.47 10.73 75.41 0.41 70.67 0.32 -0.09 4.77 5 5 5.81 5 6.31 -4.74 0.50 72.70 0.44 5.41 64.58 0.17 3.17 -8.12 -0.27 -2.24 8.43 72 1268 72 1268 72 1268 85.47 0.51 6.89 89.63 -0.12 4.89 4.16 -0.63 -2.00 4.66 2 2 86.40 0.56 7.62 2 78.44 0.23 5.60 -7.96 -0.33 -2.02 8.22 85.76 0.33 6.35 78.43 0.35 6.50 -7.33 0.02 0.15 7.33 3 85.08 0.42 6.42 4 77.21 -0.20 7.26 -7.87 -0.62 0.84 7.94 5 86.18 0.46 7.38 5 85.97 0.13 7.11 -0.21 -0.33 -0.27 0.48 0.39 85.03 6.33 78 29 -0.13 4 26 -6 74 -0.52-2 07 7.07 72 1370 72 1370 72 1370 79.17 0.47 9.09 88.85 0.01 5.14 74.80 -0.15 5.01 -14.05 -0.16 -0.13 14.05 2 78 95 0.78 9.77 88 64 0.11 3.82 85 12 -0.27 2 57 -3.52 -0.38 -1.25 3 75 2 2 73.06 0.32 7.65 83.04 -0.13 3.07 73.64 -0.432.20 -9.40 -0.30 -0.87 9.44 4 79 54 1.01 10.78 86.22 0.24 7.72 4 77.49 -0.15 6 10 -8.73 -0.39 -1.62 8.89 5 78.67 0.50 9.18 87.30 0.34 6.59 5 81.61 -0.34 3.45 -5.69 -0.68 -3.14 6.53

89.98

0.01

3.76

83.04

0.02

3.44

71.68

-0.37

3.57

-11.36

-0.39

0.13

11.37

Francisco	

Saliriai	ICISCO INC				00.4				5.00				4 100 000			
_	2-Nov-05				26-Apr-06 1075 Average L* Average a* Average b*				5-Dec-06 1075 Average L* Average a* Average b*				April 26, 2006 to December 5, 2006			
Grave	1075 A	verage L* Av	/erage a* Av	/erage b*	1075 A								ΔL* Δa		∆b*	ΔΕ
Space	1				1	68.94	-0.09	7.05	1	69.08	-0.18	4.90	0.14	-0.09	-2.15	2.16
	2				2	68.30	0.01	7.05	2	62.42	-0.01	4.82	-5.88	-0.02	-2.23	6.29
	3				3	64.14	0.33	6.85	3	70.52	-0.15	6.51	6.38	-0.48	-0.34	6.41
	4				4	67.25	-1.07	9.21	4	69.71	-0.31	5.78	2.46	0.76	-3.43	4.29
					·											
	5				5	66.83	-0.04	5.84	5	61.68	0.01	4.94	-5.15	0.05	-0.90	5.23
	6				6	62.48	0.43	7.72	6	69.71	-0.19	5.25	7.23	-0.62	-2.47	7.67
	NAWS 881 B				NAWS 881 B				NAWS 881 B							
	1	69.77	0.06	0.68	1	74.13	0.20	-0.51	1	72.41	-0.58	0.79	-1.72	-0.78	1.30	2.29
	2	67.74	0.09	0.63	2	73.78	0.17	-0.76	2	56.21	-0.16	0.95	-17.57	-0.33	1.71	17.66
	3	66.79	0.08	0.94	3	73.24	0.18	-0.42	3	68.77	-0.23	0.29	-4.47	-0.41	0.71	4.54
	4				4				4	73.62			-0.52	-0.13		
		68.57	0.06	0.76	·	74.14	0.12	0.00	•		-0.01	-0.39			-0.39	0.66
	5	69.66	0.05	1.01	5	75.10	0.12	-0.45	5	64.99	-0.32	0.55	-10.11	-0.44	1.00	10.17
	6	67.43	0.03	0.98	6	74.42	0.16	-0.52	6	59.52	0.06	1.47	-14.90	-0.10	1.99	15.03
	WS 1032 B				WS 1032 B				WS 1032 B							
	1	82.57	-0.27	2.14	1	82.80	0.00	1.48	1	76.27	-0.24	0.49	-6.53	-0.24	-0.99	6.61
	2	72.40	-0.53	3.33	2	82.49	-0.24	0.43	2	74.81	-0.29	0.45	-7.68	-0.05	0.02	7.68
	3	71.92	0.01	3.61	3	78.07	-0.33	0.52	3	68.78	-0.94	1.41	-9.29	-0.61	0.89	9.35
	4	81.78	0.03	2.63	4	85.61	0.09	1.32	4	75.38	-0.29	-0.01	-10.23	-0.38	-1.33	10.32
					·				•							
	5	81.96	-0.03	3.17	5	82.42	-0.13	0.75	5	76.98	-0.55	1.77	-5.44	-0.42	1.02	5.55
	6	81.68	0.00	2.63	6	72.06	-0.20	-0.01	6	68.90	-0.85	1.69	-3.16	-0.65	1.70	3.65
	WS 1033B				WS 1033B				WS 1033B							
	1	67.65	0.00	1.03	1	68.13	0.03	0.06	1	62.13	-0.20	-1.28	-6.00	-0.23	-1.34	6.15
	2	66.00	0.02	1.49	2	65.83	-0.12	0.20	2	61.93	-0.18	-0.89	-3.90	-0.06	-1.09	4.05
	3	67.03	0.00	1.71	3	61.40	-0.01	-0.79	3	56.71	-0.19	-0.29	-4.69	-0.18	0.50	4.72
	4	65.04	-0.09	2.21	4	69.15	0.05	0.61	4	65.13	-0.35	0.88	-4.02	-0.40	0.27	4.05
	5	67.99	-0.08	2.63	5	67.33	0.01	0.03	5	60.23	-0.12	-0.39	-7.10	-0.13	-0.42	7.11
	6	67.35		1.04	6	66.88	-0.01		6	63.98		0.49	-2.90	-0.13 -0.15	0.82	3.02
	0	67.33	0.09	1.04	0	00.00	-0.01	-0.33	О	03.90	-0.16	0.49	-2.90	-0.15	0.62	3.02
	WS 1038B				WS 1038B				WS 1038B							
						00.04	0.00	4.00	WS 1038B	74.47	0.70	0.00	0.04	0.44	0.04	0.00
	1				1	83.81	-0.32	1.69	·	74.47	-0.73	0.88	-9.34	-0.41	-0.81	9.38
	2				2	79.75	-0.72	1.53	2	71.66	-0.80	1.05	-8.09	-0.08	-0.48	8.10
	3				3	68.66	-0.76	2.97	3	64.47	-1.02	3.78	-4.19	-0.26	0.81	4.28
	4				4	81.98	-0.38	2.17	4	71.28	-0.74	1.92	-10.70	-0.36	-0.25	10.71
	5				5	78.38	-0.50	0.89	5	73.94	-0.76	1.92	-4.44	-0.26	1.03	4.57
	6				6	68.55	-0.82	2.30	6	65.53	-0.81	2.08	-3.02	0.01	-0.22	3.03
	U				U	00.55	-0.02	2.50	U	05.55	-0.01	2.00	-3.02	0.01	-0.22	5.05
	WS 862B				WS 862B				WS 862B							
	1				1	62.63	0.17	-0.78	1	63.58	-0.12	-0.83	0.95	-0.29	-0.05	0.99
	•								·							
	2				2	64.93	0.23	-0.77	2	55.19	-0.28	-0.31	-9.74	-0.51	0.46	9.76
	3				3	61.33	0.16	-1.17	3	51.74	-0.41	0.23	-9.59	-0.57	1.40	9.71
	4				4	63.87	0.23	-0.75	4	59.32	-0.10	-1.03	-4.55	-0.33	-0.28	4.57
	5				5	61.49	0.19	-0.97	5	62.15	-0.15	-1.21	0.66	-0.34	-0.24	0.78
	6				6	60.74	0.02	-0.12	6	56.63	-0.06	0.38	-4.11	-0.08	0.50	4.14
	NAWS 886B				NAWS 886B				NAWS 886B							
	1	78.87	0.03	2.86	1	81.68	-0.19	2.53	1	77.51	-0.82	3.00	-4.17	-0.63	0.47	4.24
	2	78.36	0.00	3.40	2	78.00	-0.25	1.91	2	69.91	-2.51	6.47	-8.09	-2.26	4.56	9.56
					3											
	3	80.05	0.11	2.92		69.94	-0.23	2.22	3	60.55	-2.64	9.75	-9.39	-2.41	7.53	12.28
	4	80.61	-0.13	3.40	4	79.52	-0.61	4.19	4	73.48	-1.20	5.94	-6.04	-0.59	1.75	6.32
	5	79.70	-0.39	2.34	5	71.81	-3.39	9.96	5	64.32	-3.27	8.93	-7.49	0.12	-1.03	7.56
	6	80.68	0.12	2.86	6	68.85	-1.50	6.16	6	60.50	-3.69	11.02	-8.35	-2.19	4.86	9.91
	NAWS 739B				NAWS 739B				NAWS 739B							
	1				1	70.51	0.15	1.84	1	66.30	-2.68	10.29	-4.21	-2.83	8.45	9.86
	2				2	69.67	0.22	2.32	2	65.93	-1.08	3.68	-3.74	-1.30	1.36	4.19
	3				3	69.27	0.18	2.29	3	64.35	-1.04	3.75	-4.92	-1.22	1.46	5.28
	4				4	71.69	0.11	2.37	4	67.71	-2.60	9.55	-3.98	-2.71	7.18	8.65
	5				5	69.31	0.12	2.16	5	66.16	-1.20	4.48	-3.15	-1.32	2.32	4.13
	6				6	69.27	0.13	2.14	6	63.51	-1.35	5.10	-5.76	-1.48	2.96	6.64

Color Difference for Santa Fe National Cemetery

	14-Nov-05				3-May-	3-May-06				7-Dec	7-Dec-06				May 3, 2006	to Decembe	ember 7, 2006			
Grave	U 280	Ave	rage L* Ave	rage a* Ave	rage b*	U 280	Avera	age L* A	Average a* A	verage b*	U 280	Α	verage L* A	verage a* A	verage b*	ΔL* Δ	.a* Δ	b*	ΔΕ	
Space		1					1	79.69	-0.45	4.04		1	74.96	-0.73	2.95	-4.73	-0.28	-1.09	4.86	
		2					2	74.88	-0.59	4.04		2	66.69	-0.78	5.27	-8.19	-0.19	1.23	8.28	
		3					3	72.45	-0.64	3.51		3	63.98	-0.32	5.48	-8.47	0.32	1.97	8.70	
		4					4	80.77	-0.45	2.80		4	73.80	-0.98	2.81	-6.97	-0.53	0.01	6.99	
		5					5	74.99	-0.53	2.71		5	65.98	-0.78	6.13	-9.01	-0.25	3.42	9.64	
		6					6	72.91	-0.53	2.75		6	65.48	-0.65	4.49	-7.43	-0.12	1.74	7.63	
	U 343	1	67.03	0.4	4.77	U 343	1	74.61	-0.31	6.35	U 343	1	69.57	-0.49	7.81	-5.04	-0.18	1.46	5.25	
		2	66.86	0.53	4.98		2	74.42	-0.26	6.94		2	69.60	-0.49	6.30	-4.82	-0.16	-0.64	4.86	
		3	67.96	0.33	3.72		3	73.05	-0.25	5.00		3	69.45	-0.42	4.05	-3.60	-0.10	-0.04	3.73	
		4	67.58	0.41	5.22		4	72.92	-0.25	5.89		4	70.13	-0.46	6.96	-3.60 -2.79	-0.23	1.07	3.73	
		5					5	73.89				5					-0.29		7.05	
		6	68.38	0.59	4.51		6		-0.29 -0.19	5.91		6	67.16	-0.51	8.00	-6.73		2.09		
		О	67.53	0.53	4.28		О	73.80	-0.19	4.75		О	68.66	-0.50	4.23	-5.14	-0.31	-0.52	5.18	
	U 311					U 311					U 311									
		1					1	71.69	-0.36	5.26		1	68.65	-0.58	4.04	-3.04	-0.22	-1.22	3.28	
		2					2	71.44	-0.46	4.41		2	67.94	-0.75	5.00	-3.50	-0.29	0.59	3.56	
		3					3	68.57	-0.44	4.44		3	63.59	-0.38	6.22	-4.98	0.06	1.78	5.29	
		4					4	72.35	-0.27	4.68		4	71.23	-0.57	3.88	-1.12	-0.30	-0.80	1.41	
		5					5	69.03	-0.35	5.29		5	63.77	-0.29	6.63	-5.26	0.06	1.34	5.43	
		6					6	66.78	-0.33	6.10		6	62.21	-0.37	7.30	-4.57	-0.04	1.20	4.73	
	H 530					H 530					H 530									
	П 330	1				П 330	1	76.64	0.28	9.09	П 330	1	75.34	-0.17	8.89	-1.30	-0.45	-0.20	1.39	
		2					2	76.27	0.28	10.22		2	74.59	-0.17	10.35	-1.68	-0.45	0.13	1.72	
		3					3	72.44	-0.11	9.62		3	70.81	-0.13	8.28	-1.63	-0.35	-1.34	2.16	
		4					4	75.75	0.48	9.62		4	73.69	0.01	8.95	-2.06	-0.45	-0.31	2.16	
		5					5	72.40	0.46	8.48		5	76.38	-0.13	9.63	3.98	-0.47	1.15	4.16	
		6					6	69.38	-0.16	7.52		6	68.86	-0.13	7.51	-0.52	-0.36	-0.01	0.68	
		O					O	09.30	-0.10	7.52		O	00.00	-0.00	7.51	-0.52	-0.44	-0.01	0.00	
	H 526 D					H 526 D					H 526 D									
		1	85.93	0.15	7.27		1	82.78	-0.47	3.88		1	82.18	-0.53	2.44	-0.60	-0.06	-1.44	1.56	
		2	83.52	0.04	6.49		2	81.44	-0.57	3.77		2	76.46	-0.64	2.16	-4.98	-0.07	-1.61	5.23	
		3	76.88	-0.66	9.28		3	78.70	-0.51	3.66		3	76.68	-0.61	3.12	-2.02	-0.10	-0.54	2.09	
		4	75.65	-0.59	8.37		4	88.39	-0.35	3.05		4	83.07	-0.44	3.09	-5.32	-0.09	0.04	5.32	
		5	83.57	-1.23	8.19		5	81.97	-0.42	3.16		5	76.99	-0.61	2.89	-4.98	-0.19	-0.27	4.99	
		6	83.76	-0.45	7.52		6	80.52	-0.50	3.06		6	74.40	-0.68	2.43	-6.12	-0.18	-0.63	6.15	
	H 526 J					H 526 J					H 526 J									
		1	68.98	0.34	1.94		1	77.20	0.10	0.24		1	70.18	-0.26	-0.67	-7.02	-0.36	-0.91	7.09	
		2	68.62	0.23	2.70		2	72.01	0.00	-0.45		2	70.59	-0.28	-0.53	-1.42	-0.28	-0.08	1.45	
		3	67.75	0.11	3.43		3	69.87	-0.09	-0.90		3	66.26	-0.30	-0.09	-3.61	-0.21	0.81	3.71	
		4	65.80	0.31	1.83		4	74.24	0.04	0.09		4	70.13	-0.19	0.63	-4.11	-0.23	0.54	4.15	
		5	70.15	0.28	1.93		5	71.28	-0.03	-0.51		5	70.99	-0.17	-0.66	-0.29	-0.14	-0.15	0.36	
		6	69.23	0.21	1.86		6	71.61	-0.01	-0.71		6	66.59	-0.15	0.67	-5.02	-0.14	1.38	5.21	
	1 444					1 444					1 444									
	1 444	4				1 444	4	76.15	0.40	4.50	I 444	4	72.70	0.60	4.40	2.45	0.10	0.10	2.46	
		1					1 2	76.15 72.30	-0.49 -0.46	4.59 3.79		1 2	73.70	-0.68 -0.58	4.49 4.35	-2.45	-0.19 -0.12	-0.10 0.56	2.46 6.76	
		3					3		-0.46			3	65.56 63.98		2.94	-6.74 6.16	0.12			
		3						70.14 77.15		4.49		3 4		-0.58		-6.16		-1.55	6.35	
		5					4 5		-0.52	4.48		5	68.79	-0.60	4.26	-8.36	-0.08	-0.22	8.36	
		6					5 6	73.27	-0.51 -0.58	3.27 2.54		5 6	65.28 65.82	-0.52 -0.81	4.21 2.77	-7.99 -5.39	-0.01 -0.23	0.94 0.23	8.05 5.40	
		υ					U	71.21	-0.58	2.54		О	05.02	-0.01	2.11	-5.39	-0.23	0.23	5.40	
	U 311-A					U 311-A					U 311-A									
		1	83.47	-0.18	1.82		1	81.63	-0.66	1.54		1	77.84	-1.27	2.06	-3.79	-0.61	0.52	3.87	
		2	83.37	-0.09	2.25		2	78.58	-0.79	0.97		2	52.83	-0.75	1.87	-25.75	0.04	0.90	25.77	
		3	84.39	-0.07	2.33		3	77.09	-0.65	0.21		3	66.41	-0.84	0.48	-10.68	-0.19	0.27	10.69	
		4	82.98	0.02	3.68		4	79.14	-0.54	1.28		4	76.55	-0.75	1.82	-2.59	-0.21	0.54	2.65	
		5	82.72	-0.22	2.12		5	78.21	-0.67	1.08		5	75.97	-0.94	1.98	-2.24	-0.27	0.90	2.43	
		6	83.56	-0.04	2.21		6	76.50	-0.58	1.04		6	63.66	-0.72	0.90	-12.84	-0.14	-0.14	12.84	

Appendix C. Color Analyses by Cemetery, Test Patch, and Location

:h#	1075	NAWS 881 B	WS 1032 B	NAWS 886B	WS 1033B	WS 1038B	WS 862B	NAWS 739B	Freq dE> 5	Freq dE> 1
1	2.16	2.29	6.61	4.24					1.00	0.0
2	6.29	17.66	7.68	9.56					4.00	1.0
3	6.41	4.54	9.35	12.28	Da	ata expected May 2	2007		3.00	1.0
4	4.29	0.66	10.32	6.32					2.00	1.0
5	5.23	10.17	5.55	7.56					4.00	1.0
6	7.67	15.03	3.65	9.91					3.00	1.0
									17.00	5.0
Del	Ita E for Santa I U 343	Fe H 526 D	H 526 J	U 311-A	U 280	U 311	H 530	I 444	Freq dE> 5	Freq dE> 1
1	5.25	1.56	7.09	3.87	0 200	0 311	П 330	1 444	2.00	0.0
2	4.86	5.23	1.45	25.77					2.00	1.0
3	3.73	2.09	3.71	10.69	Da	ata expected May 2	2007		1.00	1.0
4	3.00	5.32	4.15	2.65	De	ila expected May 2	2007		0.00	0.
5	7.05	4.99	0.36	2.43					1.00	0.
6	5.18	6.15	5.21	12.84					4.00	1.
o <u> </u>	0.10	0.10	0.21	12.01				_	10.00	3
	Ita E for Jeffers									
:h #	32 2904-A	32 2898-A	72 1273	72 1370	3151	3187	72 1164	72 1268	Freq dE> 5	Freq dE>
1	3.25	1.36	2.90	14.05					1.00	1
2	1.09	0.87	3.79	3.75	_				0.00	0
3	3.44	1.44	7.83	9.44	Da	ita expected May 2	2007		2.00	0.
4	3.39	15.31	2.59	8.89					2.00	1
5 6	2.42	3.98	12.92 8.98	6.53					2.00	1
o e	5.16	1.07	6.96	11.37				<u></u>	3.00 10.00	<u>1</u>
Del	Ita E for Alexan	dria								
:h#	1312	B 1200 A	C 417-A	C 418-A	B 1202	K 151	C 419	B 1201 A	Freq dE> 5	Freq dE>
1	1.92	0.11	8.38	2.84			9 110		1.00	0
2	0.69	0.67	6.53	2.37					1.00	0
3	2.08	1.69	6.24	1.60	Da	ata expected May 2	2007		1.00	0
4	1.92	3.74	6.21	0.26	20	na onpooloa may 2			1.00	0
5	3.22	3.93	6.39	1.58					1.00	0
6	4.58	2.11	5.40	3.20					1.00	0
								_	6.00	0
Del	Ita E for Bath									
h#F7		215 A	21B D	713	F 513	F 812	B 112	A1 32	Freq dE> 5	Freq dE>
1 2 3	5	ata Missing			Da	ita expected May 2	2007			

Frequency of color change by test patch for each cleaner

	Delta E for San	Francisco						
Patch #	1075	NAWS 881 B	WS 1032 B	NAWS 886B	U 343	H 526 D	H 526 J	U 311-A
D/2	2.16	2.29	6.61	4.24	5.25	1.56	7.09	3.87
Daybreak	6.29	17.66	7.68	9.56	4.86	5.23	1.45	25.77
Water	6.41	4.54	9.35	12.28	3.73	2.09	3.71	10.69
H2Orange Cleaner	4.29	0.66	10.32	6.32	3.00	5.32	4.15	2.65
WEG Marble Cleaner	5.23	10.17	5.55	7.56	7.05	4.99	0.36	2.43
Kodak Photo-flo	7.67	15.03	3.65	9.91	5.18	6.15	5.21	12.84

Delta E for Jeffe	rson Barracks								
32 2904-A	904-A 32 2898-A 72 1273			1312	B 1200 A	C 417-A	C 418-A Freq dE> 5 req dE> 10		
3.25	1.36	2.90	14.05	1.92	0.11	8.38	2.84	5.00	1.00
1.09	0.87	3.79	3.75	0.69	0.67	6.53	2.37	7.00	2.00
3.44	1.44	7.83	9.44	2.08	1.69	6.24	1.60	7.00	2.00
3.39	15.31	2.59	8.89	1.92	3.74	6.21	0.26	5.00	2.00
2.42	3.98	12.92	6.53	3.22	3.93	6.39	1.58	8.00	1.00
5.16	1.07	8.98	11.37	4.58	2.11	5.40	3.20	11.00	3.00

Frequency of color change by test patch for sunny and shady locations ${\bf Shady}$

-	Delta E for San	Francisco	Delta E for Santa Fe	elta E for Santa Fe Delta E for Jefferson Barracks Delta E for Alexandria						
Patch #	NAWS 881 B	NAWS 886B	U 343	U 311-A	32 2904-A	32 2898-A	C 417-A	C 418-A	Freq dE> 5	Freq dE> 10
D/2	2.29	4.24	5.25	3.87	3.25	1.36	8.38	2.84	2.00	0.00
Daybreak	17.66	9.56	4.86	25.77	1.09	0.87	6.53	2.37	4.00	2.00
Water	4.54	12.28	3.73	10.69	3.44	1.44	6.24	1.60	3.00	2.00
H2Orange Cleaner	0.66	6.32	3.00	2.65	3.39	15.31	6.21	0.26	3.00	1.00
WEG Marble Cleaner	10.17	7.56	7.05	2.43	2.42	3.98	6.39	1.58	4.00	1.00
Kodak Photo-flo	15.03	9.91	5.18	12.84	5.16	1.07	5.40	3.20	6.00	2.00
									22.00	8.00

Sunny	
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•	Delta E for San	Francisco	Delta E for Santa Fe Delta E for Jefferson Barracks Delta E for Alexandria							
Patch #	1075	WS 1032 B	H 526 D	H 526 J	72 1273	72 1370	1312	B 1200 A	Freq dE> 5	Freq dE> 10
D/2	2.16	6.61	1.56	7.09	2.90	14.05	1.92	0.11	3.00	1.00
Daybreak	6.29	7.68	5.23	1.45	3.79	3.75	0.69	0.67	3.00	0.00
Water	6.41	9.35	2.09	3.71	7.83	9.44	2.08	1.69	4.00	0.00
H2Orange Cleaner	4.29	10.32	5.32	4.15	2.59	8.89	1.92	3.74	3.00	1.00
WEG Marble Cleaner	5.23	5.55	4.99	0.36	12.92	6.53	3.22	3.93	4.00	0.00
Kodak Photo-flo	7.67	3.65	6.15	5.21	8.98	11.37	4.58	2.11_	5.00	1.00
								_	22.00	3.00

Appendix D. Analysis of Microorganisms on headstones in VA Cemeteries, First Report: December 2005

Analysis of Microorganisms on Headstones in VA Cemeteries



Ralph Mitchell, Kristen Bearce and Christopher McNamara

Laboratory of Applied Microbiology Division of Engineering and Applied Sciences Harvard University

December 2005

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries to provide baseline data for future testing of the effectiveness of cleaning strategies.

METHODS

Sample Collection and Study Sites

Samples were collected by Jason Church from the five cemeteries described below. Within each cemetery, samples were collected from 20 locations. A three cm² area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Samples were shipped overnight to Harvard University.

Alexandria National Cemetery

Alexandria National Cemetery is located in the community of Pineville, Rapides Parish, La. In 1804, under the new U.S. Territorial government, Rapides became one of the 12 parishes into which the Territory of New Orleans (later the State of Louisiana) was divided and, by 1805, a crude settlement had developed at the site below the rapids named Alexandria. When Abraham Lincoln was elected president in fall 1860, the people of Alexandria and Pineville saw the handwriting on the wall. On Jan. 26, 1861, the citizens of Louisiana voted for secession and swiftly committed to joining the Confederacy.

Ships appeared at the mouth of the Mississippi River determined to go upriver and capture New Orleans in May 1862. Within a year, Rapides Parish citizens were shocked when they realized their homes, the roads leading through Alexandria parish and other crossroads villages of the parish might become part of the battlefield.

Between 1863 and early 1864, the area was invaded twice. Plantations were laid waste, houses burned, fences torn down, trees cut for firewood and sugarhouses and barns burned. Both armies lived off the land, taking away food, livestock and poultry. The final destruction of Alexandria occurred on May 13, 1864, when Alexandria was burned to the ground by Union troops.

After the war, federal troops moved into the region to begin the process of reconstruction. In 1867, an eight-acre plot was appropriated from local resident François Poussin for the establishment of a national cemetery for deceased Union soldiers who died in the region. Approximately a decade later, a suit was filed by Poussin's heirs and the United States was ordered to pay his descendents \$1,200 for title to the property. Bodies were removed from the surrounding towns such as Mount Pleasant, Cheneyville and Yellow Bayou and reinterred in Alexandria. Later, remains from Fort Brown, Texas, were reinterred at the national cemetery when the fort was no longer deemed necessary. Alexandria (LA) National Cemetery was placed on the National Register of Historic Places in 1997.

The 1911 granite Memorial to Unknowns marks the burial of 1,537 unknown Federal soldiers who were removed from the Brownsville National Cemetery and re-interred at Alexandria National Cemetery. Another 1911 granite Memorial to Unknowns marks the burial of 16 unknown federal soldiers who were removed from the Fort Ringgold Post Cemetery (Texas) and re-interred at Alexandria National Cemetery. The remains of 25 unknown soldiers from post and private cemeteries near Fort Jessup, La., are also interred in one grave and it's marked with a white government marker.

There are 57 Buffalo Soldiers interred at the Alexandria National Cemetery. They represent the following units: 24th Infantry, 10th Calvary, and the 9th Calvary and are interred in Sections A, B, C, and R. ¹

¹ http://www.cem.va.gov/nchp/alexandriala.htm

Bath National Cemetery

Bath National Cemetery is located in Steuben County, N.Y., adjacent to the Department of Veterans Affairs Medical Center.

The cemetery was originally a part of the New York State Soldiers and Sailors Home, which was established in 1877; the cemetery was dedicated in Dec. 25, 1879. In 1930, the Soldiers and Sailors Home and cemetery became two integrated components of the Veterans Administration Medical Center (VAMC). When 82 national cemeteries were transferred from the Department of Army to the Veterans Administration in 1973, the Bath VAMC cemetery became part of the National Cemetery System and was designated appropriately.

Bath is the final resting place of the "first and oldest" U.S. MIAs (Missing in Action). On Oct. 26, 1987, an archeologist discovered a skeleton during the construction of a house in Fort Erie, Canada. Scientists and military historians were subsequently sent to investigate the site and ultimately, they discovered 28 remains. The bones were initially believed to be remains of the area's indigenous population. The discovery of buttons, however, led authorities to believe that the men buried at the site were British soldiers.

The 28 soldiers had been interred in a traditional manner, lying east-west with hand crossed; this indicates that they had been buried during a lull in the fighting by fellow soldiers rather than the enemy. Further investigation by the military indicated that the men had fought during the Niagara Campaign with clashes at Chippaw and Lundy's Lane before they died at Snake Hill, a battery overlooking Fort Erie. The Department of the Army, working with Canadian officials, held a repatriation ceremony at Fort Erie, Canada, on June 30, 1988 and the soldiers were reinterred with full military honors.²

Jefferson Barracks National Cemetery

Jefferson Barracks, one of the National Cemetery Administrations oldest interment sites, has served as a burial place soldiers from all wars. The original military post was built south of St. Louis, Mo., on the banks of the Mississippi River to replace Fort Bellefontaine. Selected for its strategic geographic location, the post was opened in 1826. Jefferson Barracks became the army's first permanent base west of the Mississippi River. By the 1840s, it was the largest military establishment in the United States. During the Civil War, Jefferson Barracks served as a training post for the Union Army. There was also a hospital at the post for the Union army's sick and wounded.

Although Jefferson Barracks was formally established as a national cemetery in 1866 by passage of a join resolution, the first burial, at what is now Jefferson Barracks National Cemetery is believed to have occurred the year after the post's founding, on Aug. 5, 1827. On that date, Elizabeth Ann Lash, the infant daughter of an officer stationed at Jefferson Barracks was interred at the post cemetery. The Civil War initiated the beginnings of a formal network of military cemeteries. The first general U.S. cemetery legislation was an omnibus bill enacted July 17, 1862, authorizing President Lincoln "to purchase cemetery grounds, and cause them to be securely enclosed, to be used as a national cemetery for the soldiers who shall have died in the service of the country." By the end of the year, the first 14 national cemeteries were created. Jefferson Barracks was formally established as a national cemetery in 1866 by passage of a joint resolution authorizing the Secretary of War to take action to preserve graves from desecration and "secure suitable burial-places in which they may be properly interred...."

The original portion of the cemetery is located in the northeastern section of the present acreage, appropriately delineated by four roads designated as Old Post Drive—East, West, North and South, respectively—containing Sections 1-4, and OPS-1, OPS-2, and OPS-3. It was set aside for the burial of military and civilian personnel who died at the garrison. In 1869 the cemetery experienced enormous growth when more than 10,200 recovered remains of soldiers originally buried at other Missouri locations including Cape Girardeau, Pilot Knob, Warsaw, and Rolla were removed here. About 470 victims of smallpox at Arsenal Island were also reinterred here.

The old cemetery contains approximately 20,000 gravesites, including more than 1,000 Confederate dead. During this era, Union dead were interred in sections by state, as far as that could

² http://www.cem.va.gov/nchp/bath.htm

be determined, including: 7,536 Whites, 1,067 African Americans, 1,010 Confederate POWs, and 556 "not of military service." Within the original cemetery tract, Sections 5 through 53 were laid out; the sections currently numbered 54-66, and 88, contain older burials but are irregularly numbered because the ponds, sink holes and administrative open space was converted to burial areas.

In 1870, the cemetery "quadrangle" at Jefferson Barracks measured approximately 750' x 1,230', and was surrounded by a standardized wooden picket fence "recently whitewashed." Within two years this fence was replaced by a stonewall 4,269 feet long and 1'-6" wide. A 16'-wide drive lined the interior of the wall, and crossed through the cemetery delineating large sections; narrower 10' wide paths further subdivided the grounds. "These drives and paths are covered with coarse broken stone, and, being but little used, are very uncomfortable to drive or walk over." The major interior paths had brick gutters and were lined with dense rows of the same types of trees. In addition, there were eight painted artillery guns, "planted vertically, as monuments" throughout the cemetery. In August 1871, it was reported that more than \$142,287 had been spent developing and maintaining the cemetery to date. The next year Jefferson Barracks was categorized as a "First Class" cemetery, an Army designation based on "the extent and importance" of the facilities, which also determined the superintendent's salary of \$75 per month. In 1875, the first enlargement of the cemetery took place.

During the early 1880s cast-metal tablets containing verse, "The Gettysburg Address" the War Department's General Orders No. 80, and text of the 1867 Act to establish and protect national cemeteries.

As space within the enclosure walls became limited, an expansion that would more than double the size of the cemetery was underway by the early 1890s. The original entrance with its "double iron gates hung on handsome piers of rough dressed limestone" and the old administration building/lodge were located on the north side of the existing cemetery. The landscape in some areas of Jefferson Barracks National Cemetery was one of the most contentious. Behind this building there were:

...two deep depressions in the ground, similar to the "sink-holes" in limestone formations, each having in its bottom a small pond; one has been enlarged and surrounded by a stone wall, making a miniature lake; the other is in its natural state. The ponds have subterranean communications with each other and with the Mississippi, and are affected by the rise and fall of water in that river, but are never dry.

The superintendent's personal domain included a grape arbor, privy and cistern, as well as evergreen trees and shaped planting beds of flowers and vegetables. By 1893 the approach to the entrance was established via a gravel road flanked by deciduous trees and "plank fences." Already there were a fountain, two sheds, two stables, a two-room cottage for seasonal laborers, and a rectangular rostrum (1872) located on the expanded property.

In 1922 an Executive Order assigned 170 acres of military reservation to the Veterans Bureau (now Department of Veterans Affairs). In July 1936, the War Department formally named Jefferson Barracks National Cemetery as a component of Jefferson Barracks, along with similar designations of military reservations at instillations including those named in honor of persons, target ranges and national cemeteries.

From April 1936 through the early 1940s, Depression-era government make-work programs brought improvements to the cemetery. Works Progress Administration (WPA) laborers were responsible for building 23,000' of hard-surfaced roads and walks, 46,000' concrete curbs, nearly 16,000' of "asphalt macadam" roads, and resurfacing of the same. They also removed some of the original stone wall and constructed nearly 4,600' of "common ashler (sic) stone wall, as well as miscellaneous grading. In 1946 a new stone boundary wall and entrance gate were erected. The WPA renovated the 1872 brick rostrum that measured 23'x 38' in 1941.

Gradually the importance of the post lessened and Jefferson Barracks was deactivated in 1946. Expansion of the cemetery, however, was granted by 1947 legislation authorizing the Secretary of War to "utilize and expand existing facilities" at Jefferson Barracks "when practicable, through the use of federally owned lands under the jurisdiction of the War Department" that were no longer needed for military purposes.

World War II casualties introduced a new focus to the cemetery as the central repository for group interments resulting from national disasters, when individual remains cannot be identified.

Among the more than 560 group burials—meaning two or more veterans in a common grave—are 123 victims of a 1944 Japanese massacre of POWs in the Philippines, and the remains of 41 unidentified marines who perished in a South Vietnam helicopter crash in 1968.

Jefferson Barracks National Cemetery was listed on the National Register of Historic Places in 1998.³

San Francisco National Cemetery

When Spain colonized what would become California, this area was selected as the site for a fort, or presidio, to defend San Francisco Bay. About 40 families traveled here from northern Mexico in 1776 and built the first settlement, a small quadrangle, only a few hundred feet west of what is now Funston Avenue. Mexico controlled the Presidio following 1821, but the fort became increasingly less important to the Mexican government. In 1835, most soldiers and their families moved north to Sonoma, leaving it nearly abandoned. During the Mexican War, U.S. troops occupied and repaired the damage to the fort.

The mid-century discovery of gold in California led to the sudden growth and importance of San Francisco, and prompted the U.S. government to establish a military reservation here. By executive order, President Millard Fillmore established the Presidio for military use in November 1850. During the 1850s and 1960s, Presidio-based soldiers fought Native Americans in California, Oregon, Washington and Nevada. The outbreak of the Civil War in 1861 re-emphasized the importance of California's riches and the military significance of San Francisco's harbor to the Union. This led, in 1862, to the first major construction and expansion program at the Presidio since the United States acquired it.

The Indian Wars of the 1870s and 1880s resulted in additional expansion of the Presidio, including large-scale tree planting and a post beautification program. By the following decade the Presidio had shed its frontier outpost appearance and was elevated to a major military installation and base for American expansion into the Pacific.

In 1890, with the creation of Sequoia, General Grant and Yosemite national parks in the Sierra Nevada mountains of California, the protection of these scenic and natural resources was assigned to the U.S. cavalry stationed at the Presidio. Soldiers patrolled these parks during summer months until the start of World War I in 1914. The Spanish American War in 1898 and subsequent Philippine-American War, from 1899 to 1902, increased the role of the Presidio. Thousands of troops camped in tent cities while awaiting shipment to the Philippines. Returning sick and wounded soldiers were treated in the Army's first permanent hospital, later renamed Letterman Army General Hospital. In 1914, troops under the command of Gen. John Pershing departed the Presidio for the Mexican border in pursuit of Pancho Villa and his men. When World War I began, Pershing became commander of the American Expeditionary Forces in Europe.

When the United States entered World War II after the Japanese attack on Pearl Harbor, Presidio soldiers dug foxholes along the nearby beaches. Fourth Army Commander Gen. John L. DeWitt conducted the interment of thousands of Japanese and Japanese-Americans on the West Coast while U.S. soldiers of Japanese descent were trained to read and speak Japanese at the first Military Intelligence Service language school organized at Crissy Field. During the 1950s, the Presidio served as the headquarters for the Nike missile defense program and headquarters for the famed Sixth U.S. Army. The Presidio of San Francisco, encompassing more than 350 buildings with historic value, was designated a National Historic Landmark in 1962. In 1989, the Presidio closed as a military entity and was transferred to the National Park Service in October 1994.

On Dec. 12, 1884, the War Department designated nine acres, including the site of the old post cemetery, as San Francisco National Cemetery. It was the first national cemetery established on the West Coast and, as such, marks the growth and development of a system of national cemeteries extending beyond the battlefields of the Civil War. Initial interments included the remains of the dead from the former post cemetery as well as individuals removed from cemeteries at abandoned forts and camps elsewhere along the Pacific coast and western frontier. In 1934, all unknown remains in the cemetery were disinterred and reinterred in one plot. Many soldiers and sailors who

³ http://www.cem.va.gov/nchp/jeffersonbarracks.htm

died overseas serving in the Philippines, China and other areas of the Pacific Theater are interred in San Francisco National Cemetery.

The cemetery is enclosed with a stone wall and slopes down a hill that today frames a view of the Golden Gate Bridge. Its original ornamental cast-iron entrance gates are present but have been unused since the entrance was relocated. Tall eucalyptus trees further enclose the cemetery. The lodge and rostrum date to the 1920s and reflect the Spanish Revival styling introduced to several western cemeteries.

Two unusual interments at San Francisco National Cemetery are "Major" Pauline Cushman and Miss Sarah A. Bowman. Cushman's headstone bears the inscription "Pauline C. Fryer, Union Spy," but her real name was Harriet Wood. Born in the 1830s, she became a performer in Thomas Placide's show Varieties and took the name Pauline Cushman. She married theater musician Charles Dickinson in 1853, but after her husband died of illness related to his service for Union forces, she returned to the stage. During spring 1863, while performing in Louisville, Ky., she was asked by the provost marshal to gather information regarding local Confederate activity. From there she was sent to Nashville, where she had some success conveying information about troop strength and movements. In Nashville, she was also captured and nearly hanged as a spy. She returned to the stage in 1864, to lecture and sell her autobiography. Entertainer P.T. Barnum promoted her as the "Spy of the Cumberland" and through Barnum's practiced boostership she quickly gained fleeting fame. After spending the 1870s working the redwood logging camps, she remarried and moved to the Arizona Territory. By 1893 she was divorced, destitute and desperate; she applied for her first husband's military pension and returned to San Francisco, where she died from an overdose of narcotics allegedly taken to soothe her rheumatism. Members of the Grand Army of the Republic and Women's Relief Corps conducted a magnificent funeral for the former spy. "Major" Cushman's remains reside in Officer's Circle.

Also buried at San Francisco National Cemetery is Sarah Bowman, also known as "Great Western," a formidable woman over 6 feet tall with red hair and a fondness for wearing pistols. Married to a soldier, she traveled with Zachary Taylor's troops in the Mexican War helping to care for the wounded, for which she earned a government pension. After her husband's death she had a variety of male companions and ran an infamous tavern and brothel in El Paso, Texas. Bowman left El Paso when she married her last husband. The two ended up at Fort Yuma, where she operated a boarding house until her death from a spider bite in 1866. She was given a full military funeral and was buried in the Fort Yuma Cemetery. Several years later her body was exhumed and reburied at San Francisco National Cemetery.

San Francisco National Cemetery was listed as a National Historic Landmark as part of the Presidio in 1962.⁴

Santa Fe National Cemetery

Santa Fe National Cemetery is located within the city limits of Santa Fe, N.M., approximately one mile northwest of the main plaza.

Thirteen years before the Pilgrims settled in Plymouth Colony, the Spanish had established a small settlement in Santa Fe, N.M. Santa Fe would soon become the seat of power for the Spanish Empire north of the Rio Grande and the oldest capital city in North America. Santa Fe is the site of both the oldest public building in America, the Palace of the Governors, and the nation's oldest community celebration, the Santa Fe Fiesta, established in 1712 to commemorate the Spanish reconquest of New Mexico in summer 1692. Conquistador Don Pedro de Peralta and his men laid out the plan for Santa Fe at the base of the Sangre de Cristo Mountains on the site of the ancient Pueblo ruin of Kaupoge, or "place of shell beads near the water."

When Mexico gained its independence from Spain, Santa Fe became the capital of the province of New Mexico. With the Spanish defeat came an end to the policy of a closed empire; American trappers and traders journeyed into the region along the 1,000 mile Santa Fe trail beginning in Arrow Rock, Mo. For a brief period in 1837, northern New Mexico farmers rebelled against Mexican rule, killing the provincial governor in what has been called the Chimayó

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⁴ http://www.cem.va.gov/nchp/sanfrancisco.htm

Rebellion, and occupying the capital. The insurrectionists were soon defeated and peace returned to Santa Fe for almost a decade.

In 1846, at the outset of the Mexican-American War, President James K. Polk asked General Stephen Watts Kearny to muster an army and march 1,000 miles into the Southwest to claim that region for the United States and organize territorial governments along the way. Kearny, faced with a Mexican administration weakened by years of occupation and political turmoil, was able to take Santa Fe without firing a shot. In quick succession, he won over the local leadership, assured a peaceful transition to a new civilian government and implemented a new legal code for the territory before continuing on to Arizona and California.

While there was little armed conflict in the territory of New Mexico during the Civil War, there were some engagements in the area of Santa Fe. Confederate General Henry H. Sibley raised and equipped a column to secure the secessionist claims in the New Mexico and Arizona region. Undermanned, often commanded by secessionist sympathizers and largely abandoned, the U.S. installations in the region were initially unable to defend themselves. News of the Confederate advance into New Mexico quickly raised volunteers from the Colorado Territory who took up the march. In addition, a large "California column" was raised to help defend the city of Santa Fe.

Toward the end of March 1862, Union Major John M. Chivington encountered a Confederate force southeast of the city, where the Santa Fe Trail crossed the mountains. Several days of skirmishes culminated in a battle at Glorieta Pass. Although the Confederates held their own, several hundred Union soldiers moved to the far end of the canyon and attacked the unprotected supply train. After bayoneting the pack animals and burning the wagons, the Union forces left Sibley's men little choice but to make the long trek back to Texas. The campaign not only ended Southern ambitions in the Southwest but it also forced the Confederate abandonment of Fort Bliss outside El Paso, Texas.

At the close of the Civil War, the federal government established a cemetery for the reinterment of Union soldiers who died during the brief military activity in the area. The ground initially chosen was located just west of Santa Fe and is currently part of Santa Fe National Cemetery. The Roman Catholic Diocese of Santa Fe, who owned the property, donated the land to the United States in 1870. Santa Fe's initial designation as a national cemetery was short lived. In July 1876, the War Department decided that, to save expenses, its status should be downgraded to that of a post cemetery. The superintendent was transferred to Mound City National Cemetery, Ill., and the quartermaster was transferred to Fort Macy, a local post in Santa Fe. Nine years later, however, it was re-established as a national cemetery.⁵

Enumeration of Microorganisms

Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). Photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

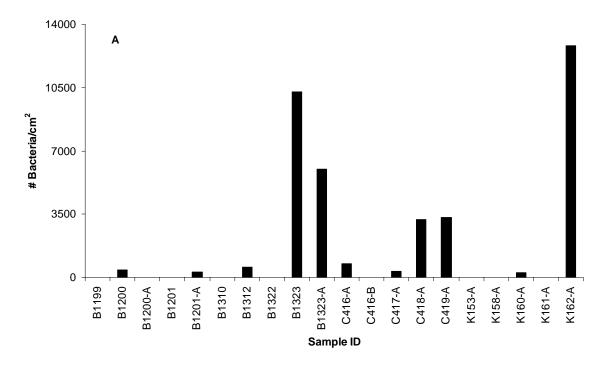
Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable (Fig. 1-5). Numbers of fungi were generally lower than bacteria. No consistent differences were found between marble types (i.e., Georgia and Colorado) or sunny and shaded areas of tombstones. Algae were not found in any samples.

⁵ http://www.cem.va.gov/nchp/santafe.htm

The number of bacteria in samples from Alexandria National Cemetery were highest in sample K162-A (Fig. 1A) while numbers of fungi were greatest in sample C416-A (Fig. 1B). Bacteria and/or fungi were present in most samples. In contrast, bacteria and fungi were detected in few samples from Bath National Cemetery (Fig. 2A and 2B). Three samples contained relatively large numbers of bacteria (A21D, D175) and fungi (A21C). Bacterial numbers in samples from Jefferson Barracks National Cemetery were highly variable (Fig. 3A). Bacteria were not detected in many samples while the highest numbers were (>17,000/cm²) were found in sample 32-2934A. Numbers of fungi were lower than bacteria, but fungi were detected in all samples except 72-1269 from Jefferson Barracks National Cemetery (Fig. 3B). Like Alexandria National Cemetery, bacteria and/or fungi were found in almost all samples. Bacteria were not detected in many samples from San Francisco National Cemetery, but the greatest number was found in sample WS1033B (Fig. 4B). Fungi were found more frequently in samples than were bacteria, and the greatest number of fungi were in samples WS1033B and WS1035A. Samples WS1033B is interesting in that this is one case in which high numbers of bacteria and fungi were found in the same sample. Numbers of bacteria and fungi were much greater in samples from Santa Fe National Cemetery than any of the other sites (note the difference in scales). Bacteria were found in most samples and numbers were highest in sample U313 (Fig. 5A). Fungi were also detected in most samples and numbers were highest in sample H526-H (Fig. 5B).

CONCLUSIONS

- Bacteria and/or fungi were found in most samples.
- Numbers of bacteria were generally greater than numbers of fungi.
- Algae were not detected in the samples.
- Our analysis of microbial growth showed wide variability in the size of the microbial community.
- Numbers of bacteria and fungi were low in most samples.
- These data will provide a useful baseline for further tests of biocide effectiveness and cleaning strategies.



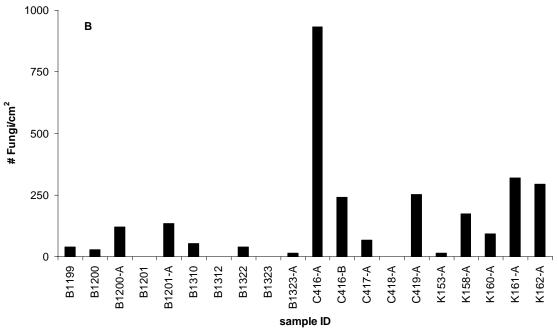
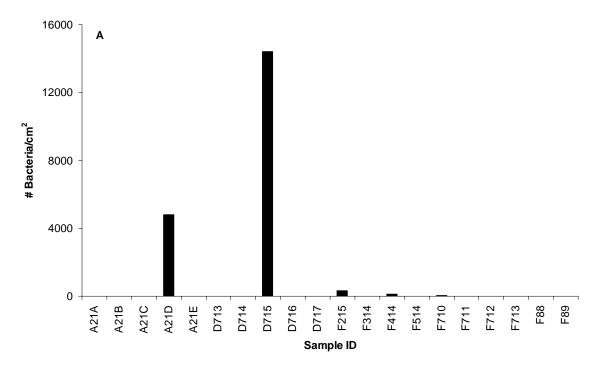


Figure 1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.



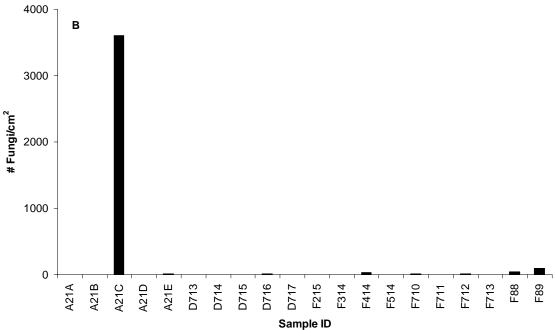
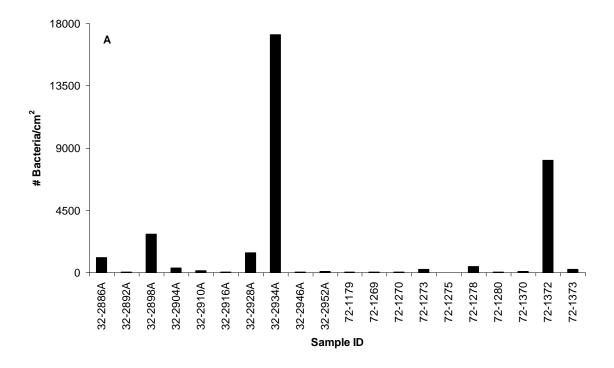


Figure 2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.



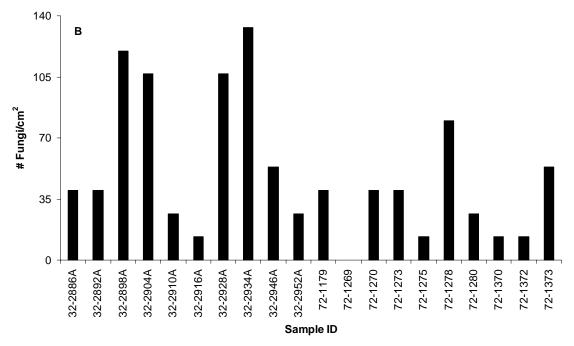
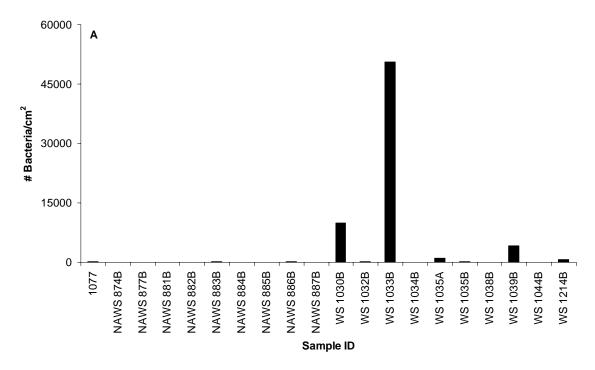


Figure 3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.



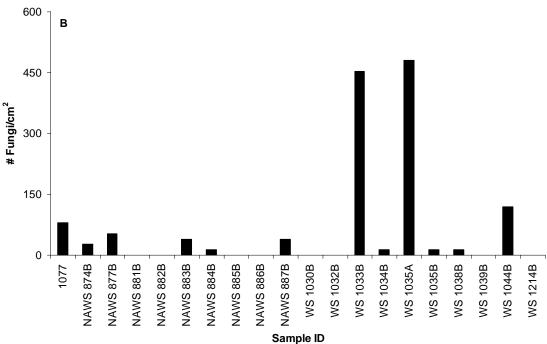


Figure 4. Numbers of bacteria (A) and fungi (B) in samples collected from San Francisco National Cemetery.

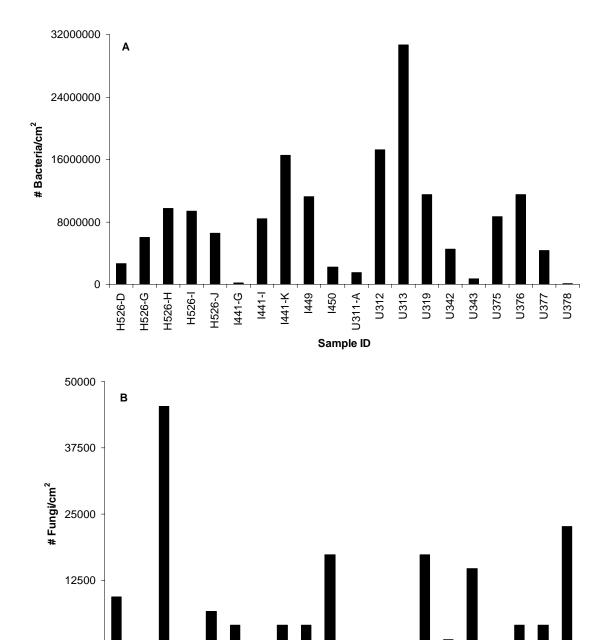


Figure 5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

U311-A

Sample ID

U312

1450

U313 U342 U375 U376

U377

1441-G

H526-G

H526-H

H526-I H526-J

APPENDIX IRaw data and calculated numbers for bacteria and fungi.

Date Collected	Cemetery location	Stone ID	Bacteria :	# Bacteria/cm ²	Funa	i # Fungi/cm²
10/4/2005	Alexandria National	B1199	0	0	3	40
10/4/2005	Alexandria National	B1200	31	413	2	27
10/4/2005	Alexandria National	B1200-A	0	0	9	120
10/4/2005	Alexandria National	B1201	0	0	0	0
10/4/2005	Alexandria National	B1201-A	22	293	1	133
10/4/2005	Alexandria National	B1310	0	0	4	53
10/4/2005	Alexandria National	B1312	44	587	0	0
10/4/2005	Alexandria National	B1322	0	0	3	40
10/4/2005	Alexandria National	B1323	77	10267	0	0
10/4/2005	Alexandria National	B1323-A	45	6000	1	13
10/4/2005	Alexandria National	C416-A	58	773	7	933
10/4/2005	Alexandria National	C416-B	0	0	18	240
10/4/2005	Alexandria National	C417-A	25	333	5	67
10/4/2005	Alexandria National	C418-A	24	3200	0	0
10/4/2005	Alexandria National	C419-A	25	3333	19	253
10/4/2005	Alexandria National	K153-A	0	0	1	13
10/4/2005	Alexandria National	K158-A	0	0	13	173
10/4/2005	Alexandria National	K160-A	19	253	7	93
10/4/2005	Alexandria National	K161-A	0	0	24	320
10/4/2005	Alexandria National	K162-A	96	12800	22	293
10/6/2005	Bath National	A21A	0	0	0	0
10/6/2005	Bath National	A21B	0	0	0	0
10/6/2005	Bath National	A21C	0	0	27	3600
10/6/2005	Bath National	A21D	36	4800	0	0
10/6/2005	Bath National	A21E	0	0	1	13
10/6/2005	Bath National	D713	0	0	0	0
10/6/2005	Bath National	D714	0	0	0	0
10/6/2005	Bath National	D715	108	14400	0	0
10/6/2005	Bath National	D716	0	0	1	13
10/6/2005	Bath National	D717	0	0	0	0
10/6/2005	Bath National	F215	24	320	0	0
10/6/2005	Bath National	F314	0	0	0	0
10/6/2005	Bath National	F414	9	120	2	27
10/6/2005	Bath National	F514	0	0	0	0
10/6/2005	Bath National	F710	2	27	1	13
10/6/2005	Bath National	F711	0	0	0	0
10/6/2005	Bath National	F712	0	0	1	13
10/6/2005	Bath National	F713	0	0	0	0
10/6/2005	Bath National	F88	0	0	3	40
10/6/2005	Bath National	F89	0	0	7	93
10/19/2005	Jefferson Barricks National	32-2886A	84	1120	3	40
10/19/2005	Jefferson Barricks National	32-2892A	4	53	3	40
10/19/2005	Jefferson Barricks National	32-2898A	21	2800	9	120
10/19/2005	Jefferson Barricks National	32-2904A	25	333	8	107
10/19/2005	Jefferson Barricks National	32-2910A	10	133	2	27
10/19/2005	Jefferson Barricks National	32-2916A	4	53	1	13

Date Collected	Cemetery location	Stone ID	Bacteria #	Bacteria/cm²	Fung	ıi # Fungi/cm²
10/19/2005	Jefferson Barricks National	32-2928A	11	1467	8	107
10/19/2005	Jefferson Barricks National	32-2934A	129	17200	1	133
10/19/2005	Jefferson Barricks National	32-2946A	4	53	4	53
10/19/2005	Jefferson Barricks National	32-2952A	8	107	2	27
10/19/2005	Jefferson Barricks National	72-1179	5	67	3	40
10/19/2005	Jefferson Barricks National	72-1269	3	40	0	
10/19/2005	Jefferson Barricks National	72-1270	4	53	3	40
10/19/2005	Jefferson Barricks National	72-1273	20	267	3	40
10/19/2005	Jefferson Barricks National	72-1275	0	0	1	13
10/19/2005	Jefferson Barricks National	72-1278	34	453	6	80
10/19/2005	Jefferson Barricks National	72-1280	3	40	2	27
10/19/2005	Jefferson Barricks National	72-1370	8	107	1	13
10/19/2005	Jefferson Barricks National	72-1372	61	8133	1	13
10/19/2005	Jefferson Barricks National	72-1373	18	240	4	53
10/19/2005	San Francisco National	1077	19	253	6	80
10/19/2005	San Francisco National	NAWS 874B	5	67	2	27
10/19/2005	San Francisco National	NAWS 877B	4	53	4	53
10/19/2005	San Francisco National	NAWS 881B	5	67	0	0
10/19/2005	San Francisco National	NAWS 882B	0	0	0	0
10/19/2005	San Francisco National	NAWS 883B	10	133	3	40
10/19/2005	San Francisco National	NAWS 884B	1	13	1	13
10/19/2005	San Francisco National	NAWS 885B	5	67	0	0
10/19/2005	San Francisco National	NAWS 886B	14	187	0	0
10/19/2005	San Francisco National	NAWS 887B	5	67	3	40
10/19/2005	San Francisco National	WS 1030B	75	10000	0	0
10/19/2005	San Francisco National	WS 1032B	14	187	0	0
10/19/2005	San Francisco National	WS 1033B	38	50667	34	453
10/19/2005	San Francisco National	WS 1034B	0	0	1	13
10/19/2005	San Francisco National	WS 1035A	8	1067	36	480
10/19/2005	San Francisco National	WS 1035B	16	213	1	13
10/19/2005	San Francisco National	WS 1038B	2	27	1	13
10/19/2005	San Francisco National	WS 1039B	31	4133	0	0
10/19/2005	San Francisco National	WS 1044B	1	13	9	120
10/19/2005	San Francisco National	WS 1214B	49	653	0	0
11/14/2005	Santa Fe National	H526-D	197	2626667	7	9333
11/14/2005	Santa Fe National	H526-G	45	6000000	4	53
11/14/2005	Santa Fe National	H526-H	73	9733333	34	45333
11/14/2005	Santa Fe National	H526-I	70	9333333	6	800
11/14/2005	Santa Fe National	H526-J	49	6533333	5	6667
11/14/2005	Santa Fe National	1441-G	11	146667	3	4000
11/14/2005	Santa Fe National	l441-l	63	8400000	0	0
11/14/2005	Santa Fe National	1441-K	124	16533333	3	4000
11/14/2005	Santa Fe National	1449	84	11200000	3	4000
11/14/2005	Santa Fe National	1450	168	2240000	13	17333
11/14/2005	Santa Fe National	U311-A	11	1466667	1	133
11/14/2005	Santa Fe National	U312	129	17200000	3	40
11/14/2005	Santa Fe National	U313	23	30666667	27	360
11/14/2005	Santa Fe National	U319	86	11466667	13	17333
11/14/2005	Santa Fe National	U342	34	4533333	1	1333

Date Collected	Cemetery location	Stone ID	Bacteria #	# Bacteria/cm ²	Fungi	# Fungi/cm²
11/14/2005	Santa Fe National	U343	54	720000	11	14667
11/14/2005	Santa Fe National	U375	65	8666667	1	133
11/14/2005	Santa Fe National	U376	86	11466667	3	4000
11/14/2005	Santa Fe National	U377	328	4373333	3	4000
11/14/2005	Santa Fe National	U378	7	93333	17	22667

Appendix E. Analysis of Microorganisms on Headstones in VA Cemeteries, Second Report: June 2006

Analysis of Microorganisms on Headstones in VA Cemeteries Second Report: June 2006



Ralph Mitchell, Kristen Bearce and Christopher McNamara

Laboratory of Applied Microbiology Division of Engineering and Applied Sciences Harvard University

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries six months after cleaning.

RESULTS FROM PREVIOUS SAMPLE COLLECTION

Bacteria and/or fungi were found in most samples collected in October and November 2005 (see Appendix 1). Numbers of bacteria were generally greater than numbers of fungi and algae were not detected in the samples. Our analysis of microbial growth showed wide variability in the size of the microbial community. However, numbers of bacteria and fungi were low in most samples.

METHODS

Sample Collection and Study Sites

Samples were collected during April and May 2006 by Jason Church from five cemeteries: 1) Alexandria National Cemetery, Alexandria, VA, 2) Bath National Cemetery, Steuben County, NY, 3) Jefferson Barracks National Cemetery, St. Luois, MO, 4) San Francisco National Cemetery, San Francisco, CA, and 5) Santa Fe National Cemetery, Santa Fe, NM. Within each cemetery, samples were collected from 20 locations. A three cm² area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Sample locations were cleaned in October and November 2005 using five different agents: Daybreak, 5914 (NCH Corporation, Irving, TX), Marble and Granite Cleaner Concentrate (World Environmental Group, Inc., Ocala, FL), Photo-Flo 200 (Eastman Kodak Company, Rochester, NY), H₂Orange₂ Grout Safe (EnvirOx LLC, Danville, IL), and D/2 Architectural Antimicrobial (Sunshine Makers, Inc., Huntington Harbour, CA). Samples were stored un-refrigerated for a number of days prior to shipment to Harvard University. Samples were shipped overnight to Harvard University.

Enumeration of Microorganisms

Samples collected from the headstones were enumerated (Table 1). Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). When present, photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

Large numbers of bacteria were found in samples from Alexandria National Cemetery (Fig. 1A). The largest number of bacteria were found in the sample from the sun-exposed location cleaned with Photoflow. The smallest number of bacteria was found in the shaded location sample location cleaned with Marble/Granite cleaner. Numbers of fungi from Alexandria National Cemetery were much lower than numbers of bacteria (Fig. 1B). The smallest numbers of fungi were found in samples cleaned with Daybreak while the largest number of fungi was found in the shaded location cleaned with D2.

Numbers of bacteria from Bath National Cemetery were very high in all samples, and were greatest in the shaded sample cleaned with Photoflow (Fig. 2A). The lowest number of bacteria was found in the sun-exposed sample cleaned with Photoflow. Numbers of fungi in samples from Bath National cemetery were much lower than number of bacteria, but were greater than the numbers of fungi found in Alexandria (Fig. 2B). The greatest numbers of fungi were found in the locations cleaned with D2. Fungi were below the detection limit in the shaded sample cleaned with Daybreak.

Numbers of bacteria in samples from Jefferson National Cemetery were greatest in sunexposed samples cleaned with H₂ Orange and Photoflow (Fig. 3A). The lowest numbers of bacteria were found in samples cleaned with D2, Daybreak, and Marble/Granite Cleaner. Numbers of fungi were generally higher in shaded locations (fig. 3B). The lowest numbers of fungi were found in samples cleaned with Marble/Granite cleaner.

The lowest number of bacteria at San Francisco National Cemetery were found in sunexposed locations cleaned with Daybreak (Fig. 4A). Large numbers of bacteria were found in all other samples from this cemetery. Numbers of fungi were extremely variable. The lowest numbers of fungi were found in locations treated with H₂ Orange (Fig. 4B). The highest numbers of fungi were observed in samples cleaned with D2 and Photoflow.

Numbers of bacteria in samples from Santa Fe National Cemetery were generally higher in shaded locations than in sun-exposed areas (Fig. 5A). The lowest numbers of bacteria were found in samples from sun-exposed locations cleaned with Daybreak and Marble/Granite cleaner. Numbers of fungi were quite low, with the exception of the shaded location cleaned with Marble/Granite cleaner (Fig. 5B). Fungi were below detection limits in the sun-exposed location cleaned with D2 and the shaded location cleaned with Photoflow.

CONCLUSIONS

- Large numbers of bacteria and fungi were found in all samples.
- The large numbers of microorganisms enumerated is inconsistent with visual observations made by Jason Church, in which locations cleaned with D2 and Daybreak appeared to be free from microbial growth.
- Inconsistencies between visual observations and microbial counts may be due to growth of microorganisms in the swabs after sampling.
- The swabs used in this study contain Amies medium to prolong survival of the microorganisms during transport. Because swabs were stored for long periods of time without refrigeration before shipment to Harvard, growth of microorganisms may have occurred.
- We recommend that future samples be shipped to Harvard on ice immediately after collection.

Table 1. Samples enumerated in this study.

Cemetery	Stone Identifier	Environment	Marble Type
Alexandria	C417A	Shady	Colorado
Alexandria	B1312	Sunny	Colorado
Bath	D713	Shady	Colorado
Bath	D714	Shady	Colorado
Bath	D715	Shady	Colorado
Bath	F710	Sunny	Colorado
Bath	F711	Sunny	Colorado
Bath	F712	Sunny	Colorado
Jefferson	32-2886A	Shady	Colorado
Jefferson	32-2904A	Shady	Colorado
Jefferson	32-2928A	Shady	Colorado
Jefferson	72-1269	Sunny	Colorado
Jefferson	72-1270	Sunny	Colorado
Jefferson	72-1370	Sunny	Colorado
San Francisco	NAWS 886 B	Shady	Colorado
San Francisco	WS 1032 B	Sunny	Colorado
Santa Fe	U311-A	Shady	Colorado
Santa Fe	H 526 D	Sunny	Colorado

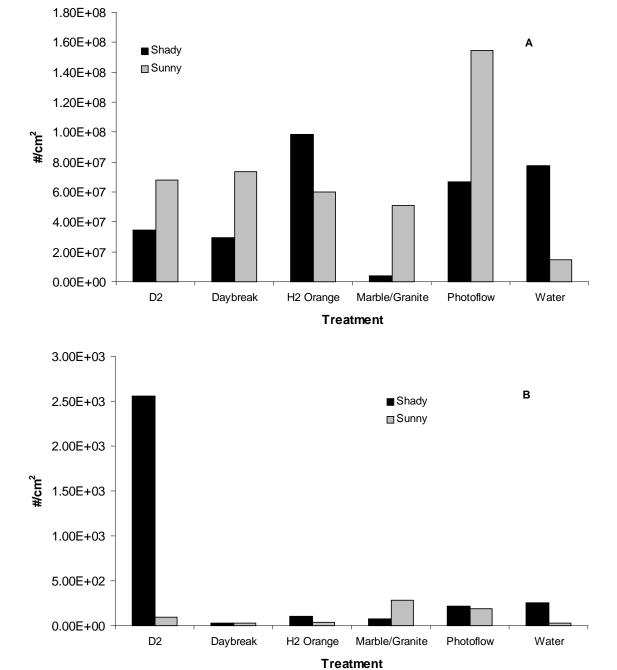


Figure 1. Numbers of bacteria (A) and fungi (B) in samples from Alexandria National Cemetery.

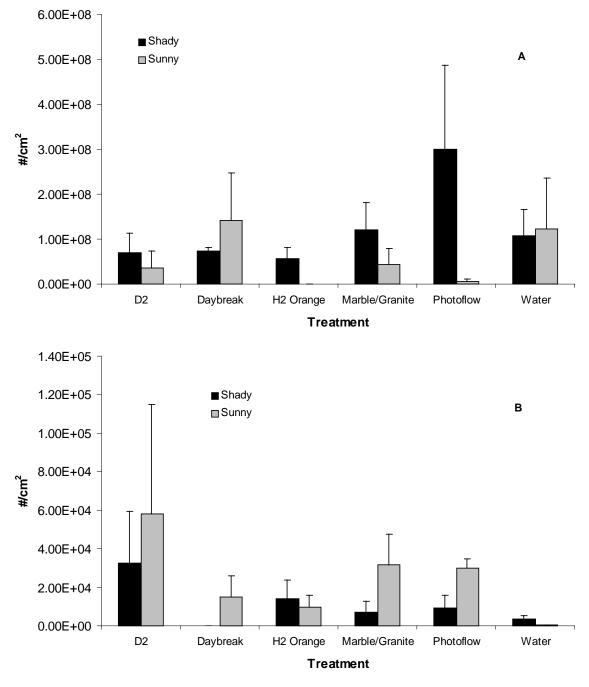


Figure 2. Numbers of bacteria (A) and fungi (B) in samples from Bath National Cemetery.

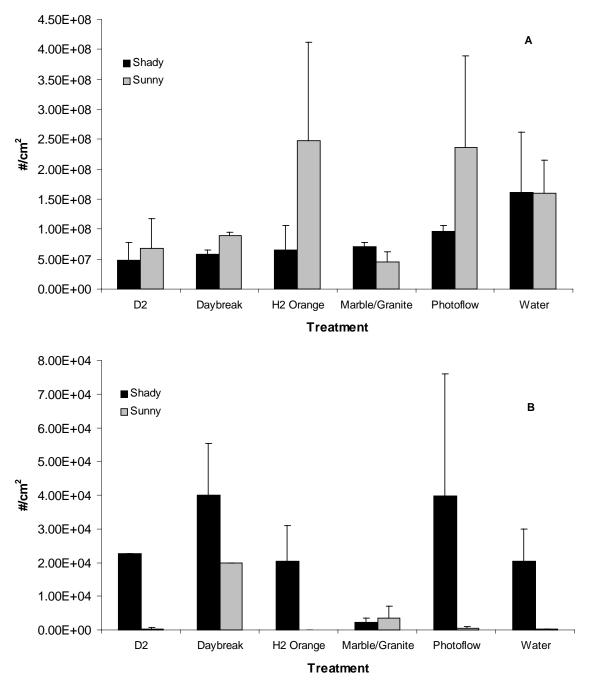


Figure 3. Numbers of bacteria (A) and fungi (B) in samples from Jefferson Barracks National Cemetery.

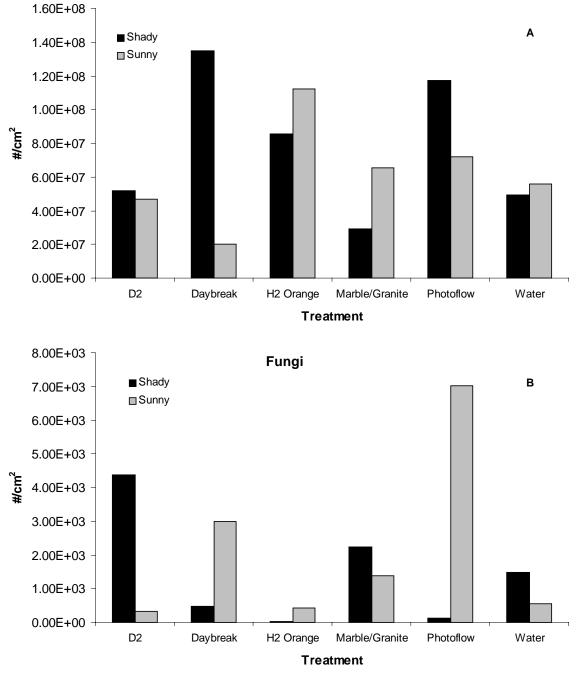


Figure 4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

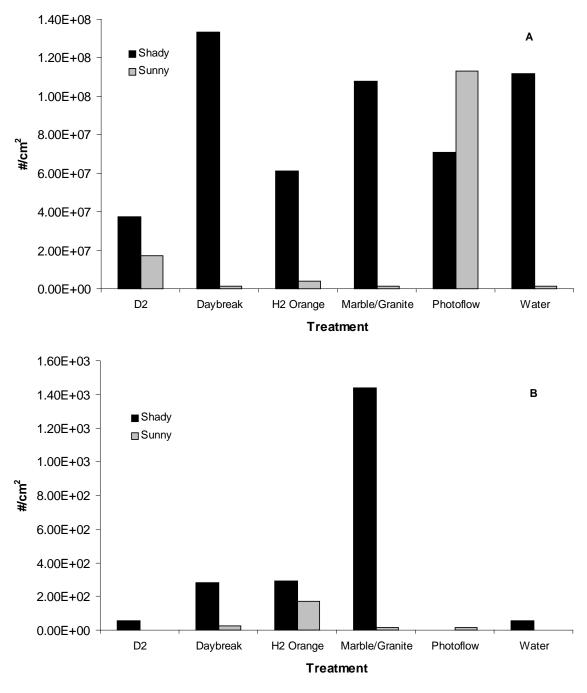
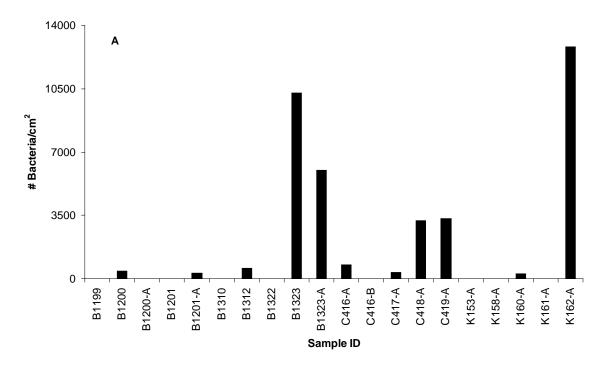


Figure 5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

APPENDIX 1. Results of Initial Samples Collected in October - November 2005



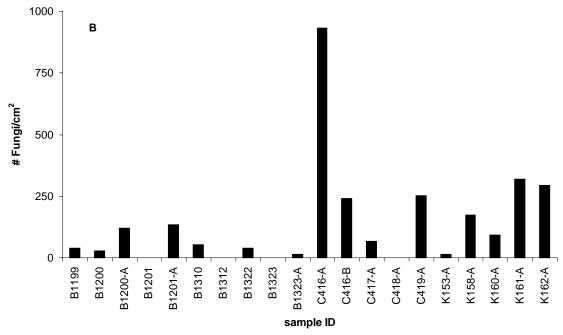
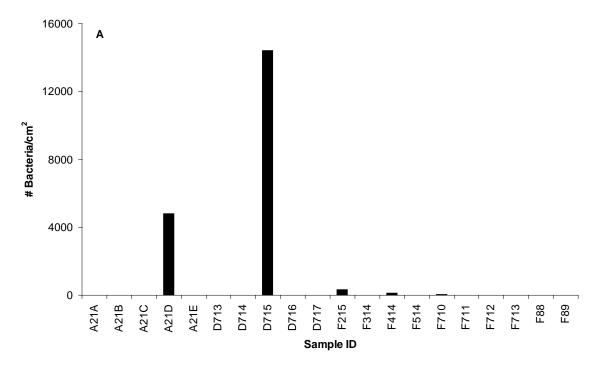


Figure 1-1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.



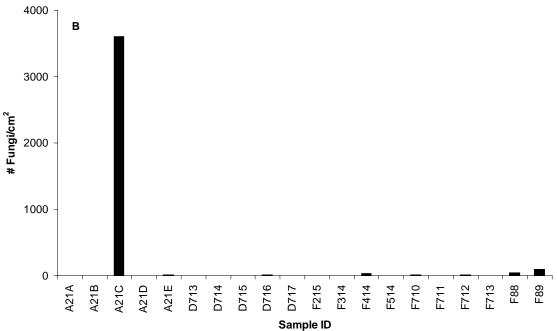
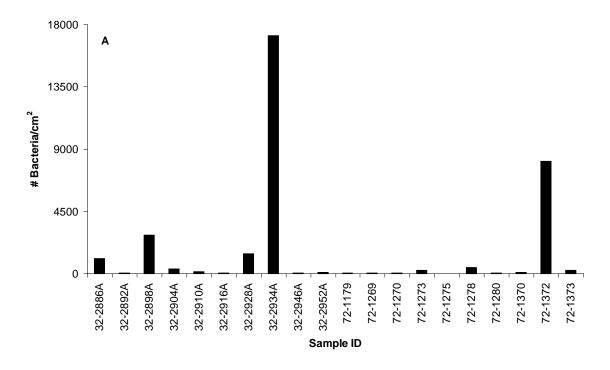


Figure 1-2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.



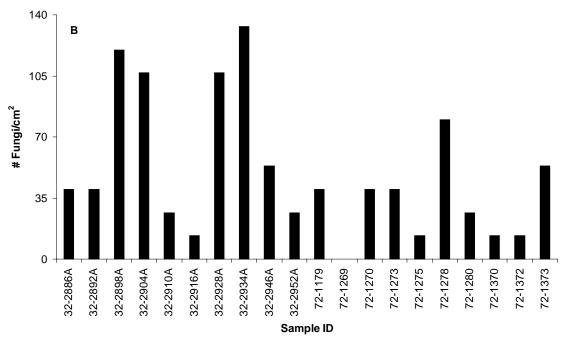
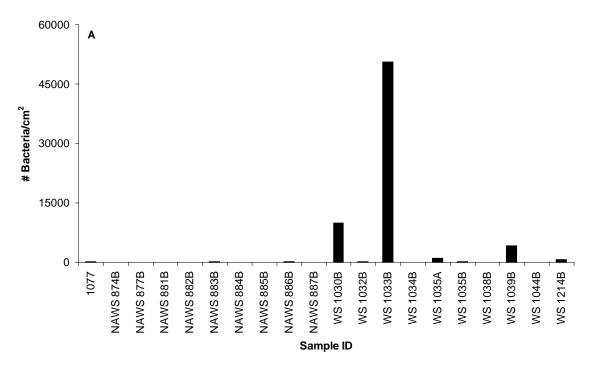


Figure 1-3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.



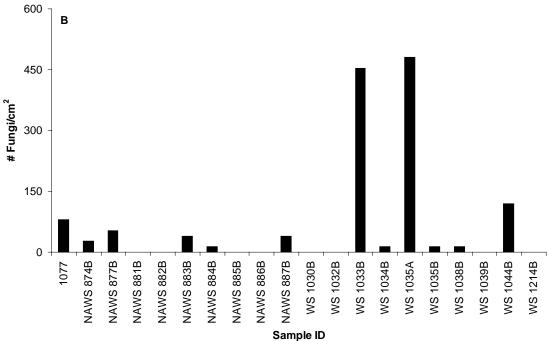


Figure 1-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

Appendix F. Analysis of Microorganisms on Headstones in VA Cemeteries, Third Report: February 2007

Analysis of Microorganisms on Headstones in VA Cemeteries Third Report: February 2007



Ralph Mitchell, Kristen Bearce Lee and Christopher McNamara

Laboratory of Applied Microbiology Division of Engineering and Applied Sciences Harvard University

SUMMARY

- Few differences were observed between locations cleaned in 2005 and 2006, suggesting that the cleaning agents' protection against bacterial and fungal re-growth is short, probably less than one year.
- No algae or photosynthetic bacteria were observed in the samples.
- The absence of algae or photosynthetic bacteria is significant. These organisms typically provide the most visual evidence of growth on headstones. Their absence, even from the stones treated with water, suggests it is still too early to determine the effectiveness of the biocides.
- Numbers of bacteria were generally greater than numbers of fungi.
- No consistent differences in numbers of bacteria or fungi were found among the cleaning agents.
- The most consistent differences were observed between sunny and shaded locationsabundance of bacteria and fungi were frequently greater in shady locations. This is most likely due to drier conditions and more intense UV irradiation in sunny locations.

OBJECTIVES

The objective of this project is to test cleaning agents for use in cleaning headstones within national cemeteries overseen by the National Cemetery Administration. The purpose of the current work was to analyze of numbers of microorganisms in samples collected from tombstones in five Veterans Administration cemeteries one year after cleaning.

RESULTS FROM PREVIOUS SAMPLE COLLECTION

Fall 2005 samples

Bacteria and/or fungi were found in most samples collected in October and November 2005 (see Appendix 1). Numbers of bacteria were generally greater than numbers of fungi. Algae were not detected in the samples. Our analysis of microbial growth showed wide variability in the size of the microbial community. However, numbers of bacteria and fungi were low in most samples.

Spring 2006 Samples

Large numbers of bacteria and fungi were found in all samples (see Appendix 2). The large numbers of microorganisms enumerated were inconsistent with visual observations made by Jason Church, in which locations cleaned with D2 and Daybreak appeared to be free from microbial growth. Inconsistencies between visual observations and microbial counts may have been due to growth of microorganisms in the swabs after sampling.

METHODS

Sample Collection and Study Sites

Samples were collected during November and December 2006 by Jason Church from five cemeteries: 1) Alexandria National Cemetery, Pineville, LA 2) Bath National Cemetery, Steuben County, NY, 3) Jefferson Barracks National Cemetery, St. Louis, MO, 4) San Francisco National Cemetery, San Francisco, CA, and 5) Santa Fe National Cemetery, Santa Fe, NM. A three cm² area of the tombstones were sampled for microorganisms using BBL Culture Swabs (Becton-Dickinson, Sparks, MD). Sample locations were cleaned in either 2005 or 2006 using five different agents: Daybreak, 5914 (NCH Corporation, Irving, TX), Marble and Granite Cleaner Concentrate (World Environmental Group, Inc., Ocala, FL), Photo-Flo 200 (Eastman Kodak Company, Rochester, NY), H₂Orange₂ Grout Safe (EnvirOx LLC, Danville, IL), and D/2 Architectural Antimicrobial (Sunshine Makers, Inc., Huntington Harbour, CA). In this round of samples, locations cleaned with D/2, Daybreak, Marble and Granite Cleaner, and water were sampled. Samples were shipped overnight to Harvard University.

Enumeration of Microorganisms

Samples collected from the headstones were enumerated (Table 1). Bacteria and fungi were enumerated by plating samples on solid media. Plates were incubated at room temperature for two days and colonies were counted. Bacteria were plated on Difco Nutrient Agar (Becton-Dickinson, Sparks, MD) and fungi were plated on malt extract agar (6.4 g/L maltose, 1.4 g/L dextrose, 1.2 g/L glycerol, 0.4 g/L peptone, 7.5 g/L agar, 4875 U penicillin G, 3250 U bacitracin). When present, photosynthetic microorganisms (algae) were analyzed using a hemocytometer. The numbers of algae in at least 10 fields of view were counted at 40X magnification.

RESULTS

No algae were detected in samples from any of the five cemeteries sampled. Green coloration in some samples was due to the presence of fungi. Fungi and bacteria were enumerated by plating on solid media and counting colonies after incubation. Numbers of bacteria and fungi in samples were variable.

Large numbers of bacteria were found in samples from Alexandria National Cemetery (Fig. 1A). The largest numbers of bacteria were found in samples cleaned with D/2 (Shady 2006 and Sunny 2005). The smallest number of bacteria was found in the shaded location sample location cleaned with Marble/Granite cleaner. Numbers of fungi from Alexandria National Cemetery were more variable than numbers of bacteria, but frequently were as abundant as the bacteria (Fig. 1B). No fungi were observed in sunny locations cleaned in 2005.

Bacteria in samples from Bath National Cemetery were high in the shady locations and generally low or not found in sunny locations (Fig. 2A). There did not appear to be any differences between cleaning agents. As was found in Alexandria, numbers of fungi were much more variable than bacteria at Bath National Cemetery (Fig. 2B). For all cleaning agents except Daybreak, numbers of fungi were greater shady locations than sunny locations.

Numbers of bacteria in samples from Jefferson Barracks National Cemetery were variable and there were no consistent differences between cleaning agents (Fig. 3A). The lowest numbers of bacteria were found in sunny locations cleaned with Daybreak. Numbers of fungi were generally higher in shaded locations (Fig. 3B). Sunny locations cleaned in 2006 had the lowest numbers of fungi.

The numbers of bacteria were generally very high in samples from San Francisco National Cemetery (Fig. 4A). Again, there were no consistent differences between cleaning agents. Numbers of fungi were variable, but much lower than numbers of bacteria (Fig. 4B). No fungi were observed in sunny locations cleaned with Daybreak.

Numbers of bacteria in samples from Santa Fe National Cemetery were generally high (Fig. 5A). In most cases numbers were greater in samples from shaded locations than sunny locations. The lowest numbers of bacteria were found in samples from sunexposed locations cleaned with Daybreak in 2006. Numbers of fungi were fairly consistent, with most samples having about 10,000 colony forming units/cm² (Fig. 5B). Like the bacteria, the lowest number of fungi was found in the locations cleaned with Daybreak in 2006.

Table 1. Samples enumerated in this study.

Cemetery	Stone Identifier	Environment	Year Cleaned	Marble Type
Alexandria	C 417-A	Shady	2005	Colorado
Alexandria	C 419	Shady	2006	Colorado
Alexandria	В 1312	Sunny	2005	Colorado
Alexandria	B 1202	Sunny	2006	Colorado
Bath	D 7 13	Shady	2005	Colorado
Bath	B1 11	Shady	2006	Colorado
Bath	F 7 12	Sunny	2005	Colorado
Bath	F 8 12	Sunny	2006	Colorado
Jefferson	32 2904-A	Shady	2005	Colorado
Jefferson	3187	Shady	2006	Colorado
Jefferson	72 1370	Sunny	2005	Colorado
Jefferson	72 1268	Sunny	2006	Colorado
San Francisco	NAWS 886B	Shady	2005	Colorado
San Francisco	1075	Shady	2006	Colorado
San Francisco	WS 1032B	Sunny	2005	Colorado
San Francisco	WS 1038B	Sunny	2006	Colorado
Santa Fe	U 311-A	Shady	2005	Colorado
Santa Fe	U 280	Shady	2006	Colorado
Santa Fe	H 526 D	Sunny	2005	Colorado
Santa Fe	H 530	Sunny	2006	Colorado

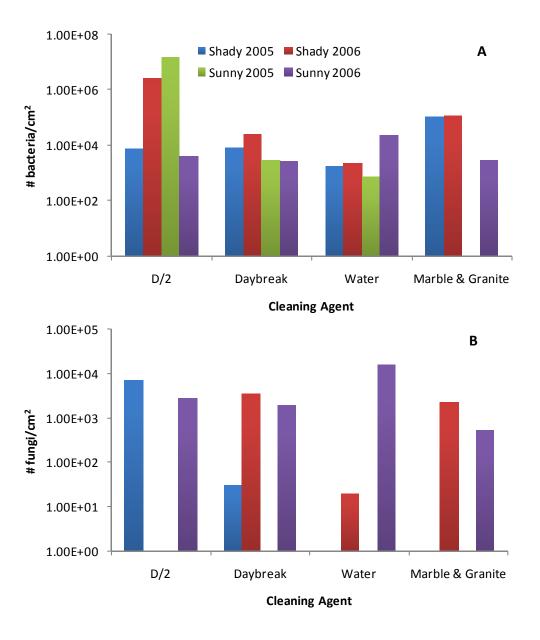


Figure 1. Numbers of bacteria (A) and fungi (B) in Alexandria National Cemetery samples from locations cleaned in 2005 and 2006.

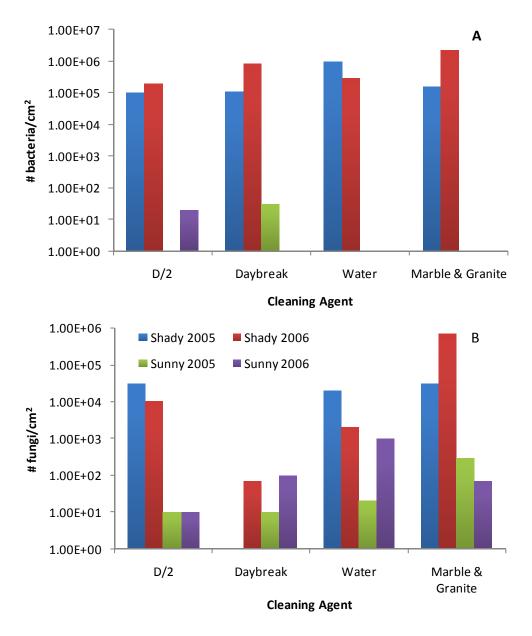


Figure 2. Numbers of bacteria (A) and fungi (B) in Bath National Cemetery samples from locations cleaned in 2005 and 2006.

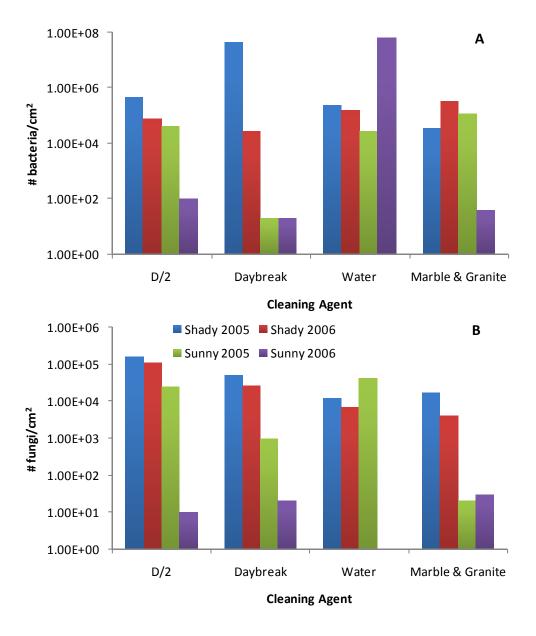


Figure 3. Numbers of bacteria (A) and fungi (B) in Jefferson Barracks National Cemetery samples from locations cleaned in 2005 and 2006.

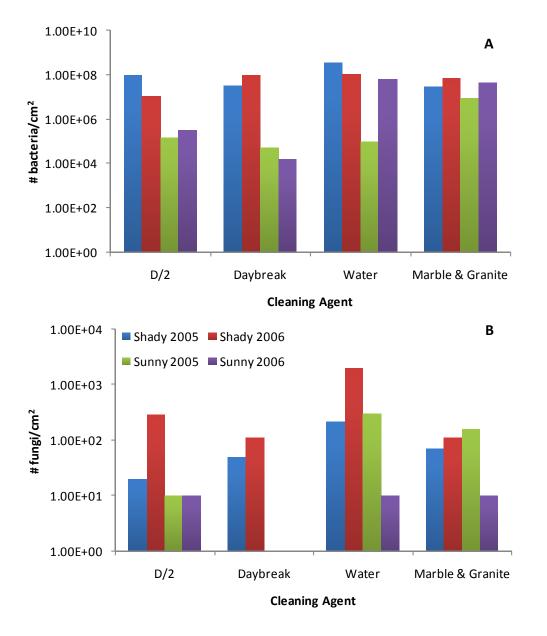


Figure 4. Numbers of bacteria (A) and fungi (B) in San Francisco National Cemetery samples from locations cleaned in 2005 and 2006.

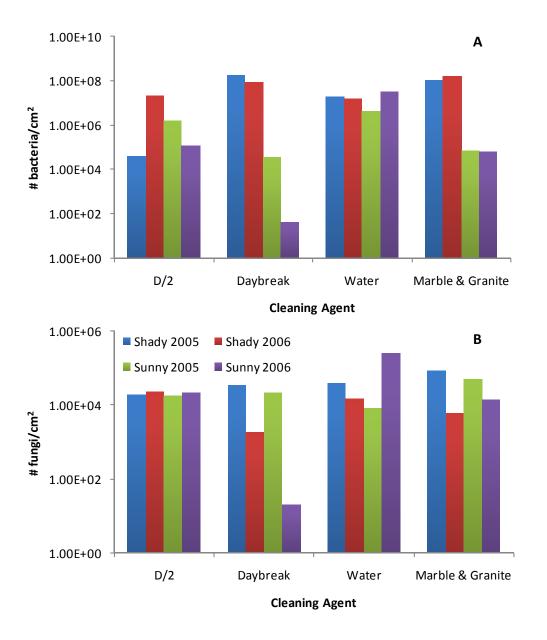
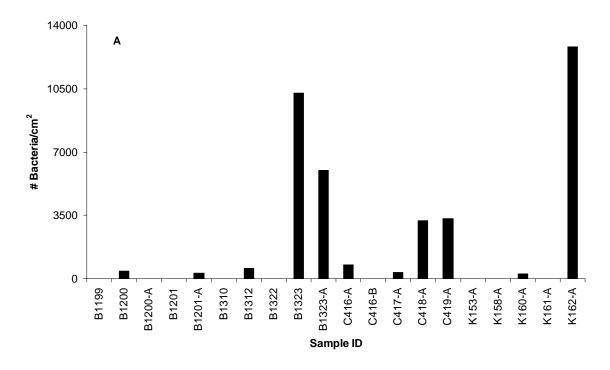


Figure 5. Numbers of bacteria (A) and fungi (B) in Santa Fe National Cemetery samples from locations cleaned in 2005 and 2006.

APPENDIX 1

Results of Initial Samples Collected in October – November 2005



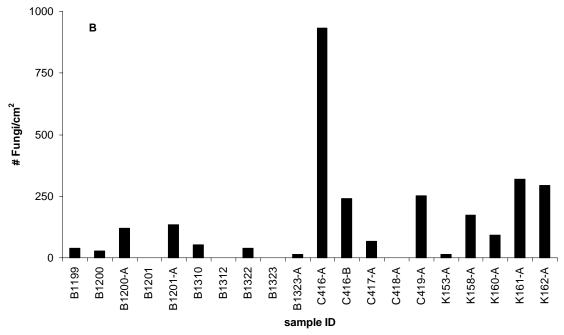
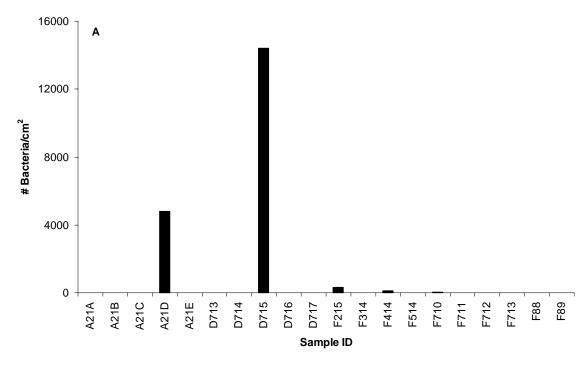


Figure 1-1. Numbers of bacteria (A) and fungi (B) in samples collected from Alexandria National Cemetery.



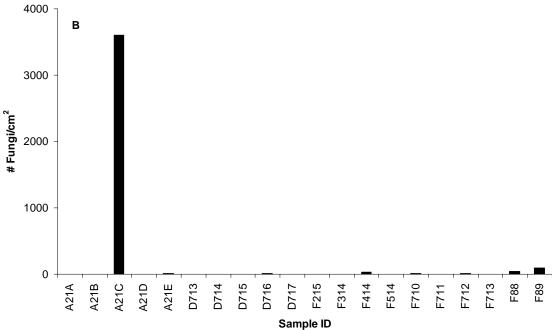
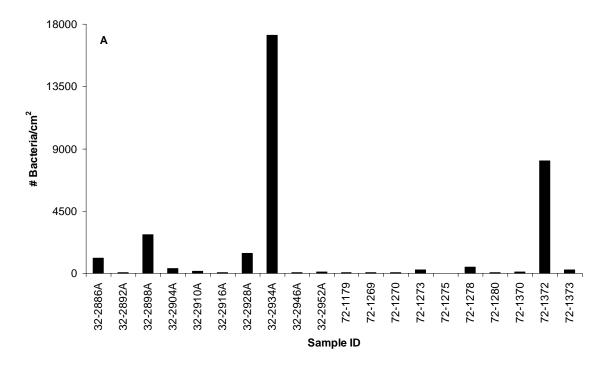


Figure 1-2. Numbers of bacteria (A) and fungi (B) in samples collected from Bath National Cemetery.



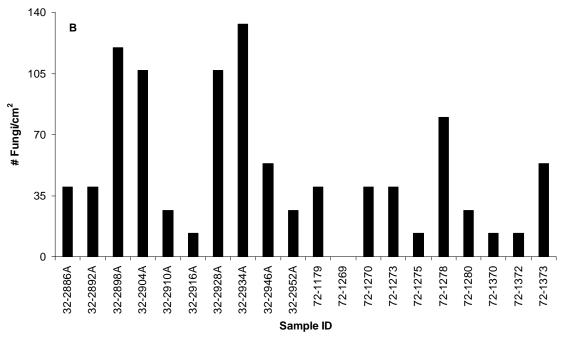


Figure 1-3. Numbers of bacteria (A) and fungi (B) in samples collected from Jefferson Barracks National Cemetery.

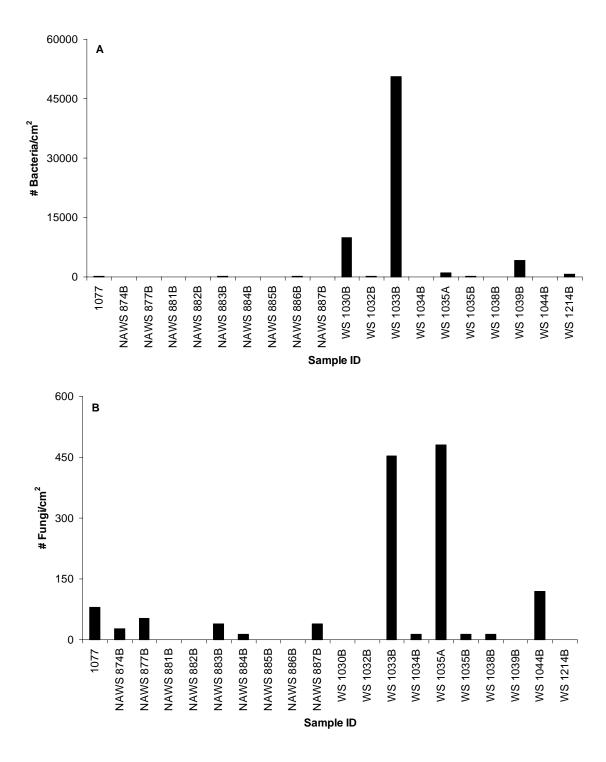


Figure 1-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

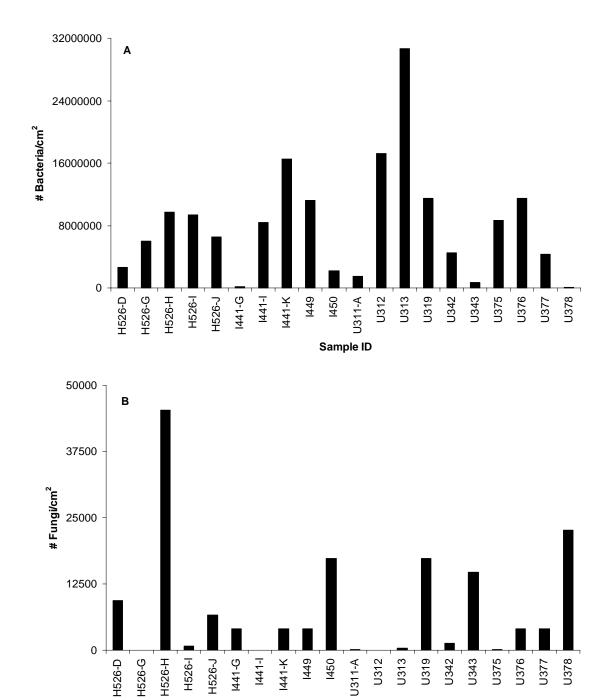
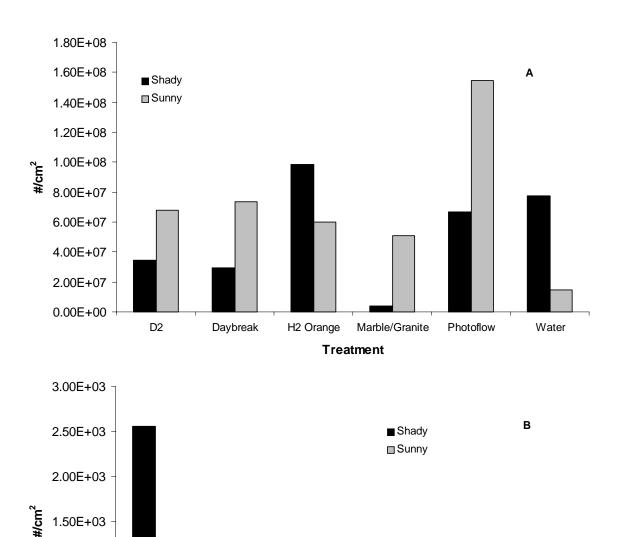


Figure 1-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

Sample ID

APPENDIX 2

Results of samples collected in April-May 2006



D2 Daybreak H2 Orange Marble/Granite Photoflow Water

Treatment

Figure 2-1. Numbers of bacteria (A) and fungi (B) in samples from Alexandria National Cemetery.

1.00E+03

5.00E+02

0.00E+00

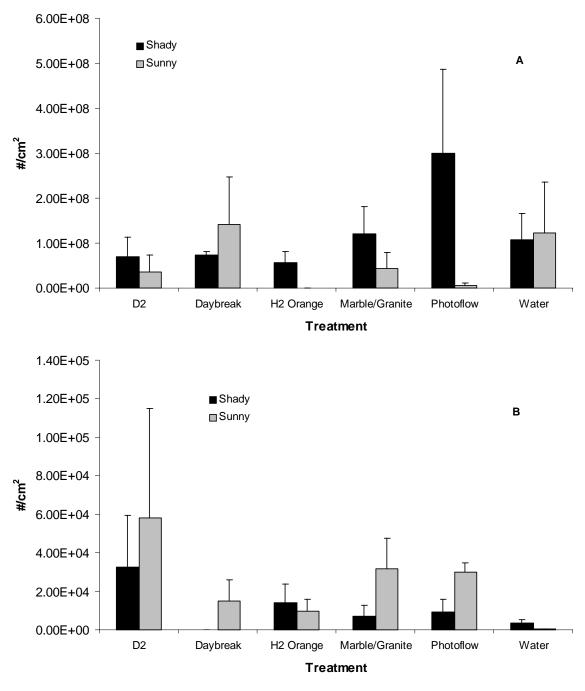


Figure 2-2. Numbers of bacteria (A) and fungi (B) in samples from Bath National Cemetery.

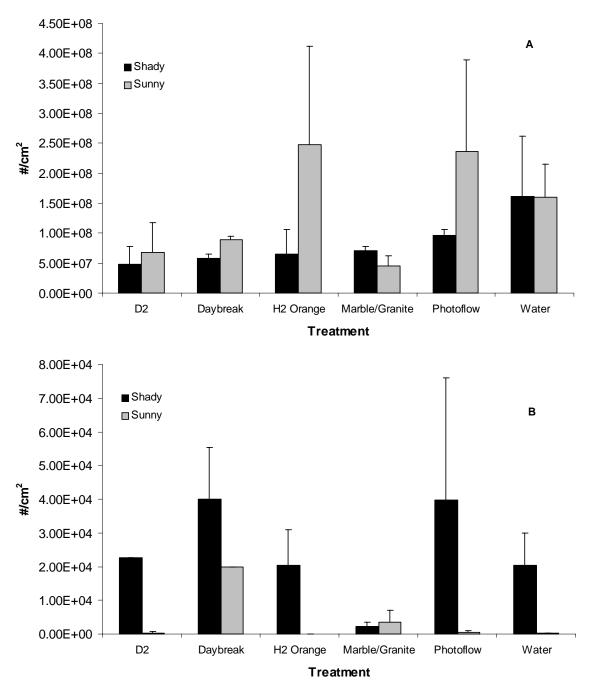


Figure 2-3. Numbers of bacteria (A) and fungi (B) in samples from Jefferson Barracks National Cemetery.

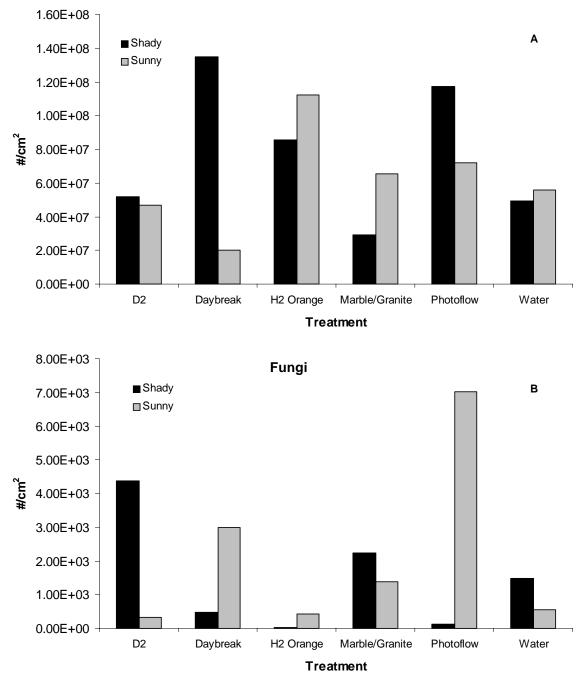


Figure 2-4. Numbers of bacteria (A) and fungi (B) in samples from San Francisco National Cemetery.

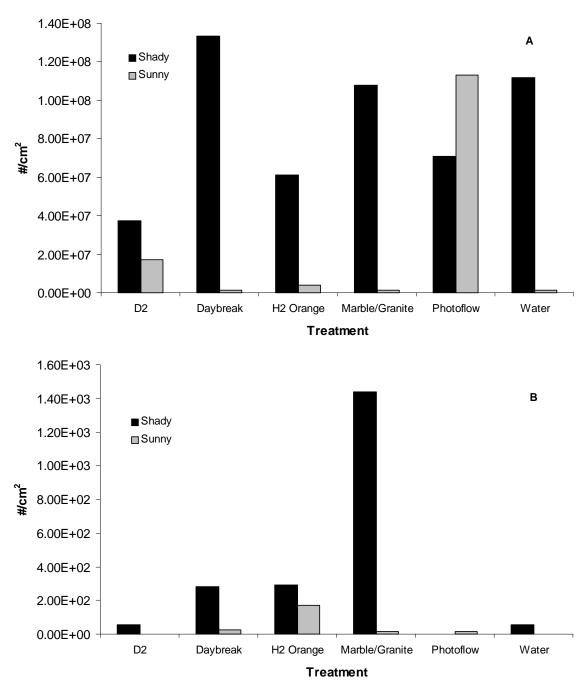


Figure 2-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

Appendix G. Biological Performance Based on June 2006 Report

Appendix, Biolog	ical Perforr	nance Bas	ed on Sun	or Shade									
	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	Shade Overall	Bshade	Fshade
D2	4	1	4	1	5	3	4	1	4	6	3.3	4.2	2.4
Daybreak	5	6	4	6	4	1	1	5	1	5	3.8	3	4.6
H2Orange	1	6	5	3	4	3	3	6	3	5	3.9	3.2	4.6
Marble/Granite	6	6	3	4	4	6	5	3	2	1	4	4	4
Photo-flo	3	5	1	4	3	1	2	6	3	6	3.4	2.4	4.4
Water	2	5	3	5	1	3	4	4	2	6	3.5	2.4	4.6
	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	Sun Overall	Bsun	Fsun
D2	3	4	4	1	4	6	3	6	5	6	4.2	3.8	4.6
Daybreak	3	6	1	4	3	1	5	3	6	4	3.6	3.6	3.6
H2Orange	3	5	6	5	1	6	1	6	6	1	4	3.4	4.6
Marble/Granite	4	1	4	3	5	5	2	4	6	5	3.9	4.2	3.6
Photo-flo	1	2	6	3	1	6	2	1	1	5	2.8	2.2	3.4
Water	6	6	2	6	2	6	3	5	6	6	4.8	3.8	5.8
	TotGrowth		TotGrowth	TotGrowth	TotGrowth		TotGrowth	TotGrowth	TotGrowth	TotGrowth		Boverall	Foverall
D2	3.5	2.5	4	1	4.5	4.5	3.5	3.5	4.5	6	3.75	4	3.5
Daybreak	4	6	2.5	5	3.5	1	3	4	3.5	4.5	3.7	3.3	4.1
H2Orange	2	5.5	5.5	4	2.5	4.5	2	6	4.5	3	3.95	3.3	4.6
Marble/Granite	5	3.5	3.5	3.5	4.5	5.5	3.5	3.5	4	3	3.95	4.1	3.8
Photo-flo	2	3.5	3.5	3.5	2	3.5	2	3.5	2	5.5	3/	2.3	3.9
Water	4	5.5	2.5	5.5	1.5	4.5	3.5	4.5	4	6	4.15	3.1	5.2

Rankings from 1-6, ties allowable

(lower numbers indicates worse performance)

Appendix H. Biological Performance Based on February 2007 Report

Appendix H, Biological Performance Based on Sun or Shade based on Biological Analyses February 2007

	ANC		BNC		JBNC		SFNC		SFeNC				
	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	Shade Overall	Bshade	Fshade
D2	1	4	4	2	3	1	4	2	3	1	2.5	3	2
Daybreak	4	1	2	4	4	2	1	3	1	4	2.6	2.4	2.8
Marble/Granite	3	3	3	3	2	3	2	1	4	2	2.6	2.8	2.4
Water	2	2	1	1	1	4	3	4	2	3	2.3	1.8	2.8
	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	Sun Overall	Bsun	Fsun
D2	3	2	1	4	2	3	3	3	3	2	2.6	2.4	2.8
Daybreak	4	3	4	2	4	2	4	4	4	4	3.5	4	3
Marble/Granite	2	1	4	1	1	4	1	3	1	1	1.9	1.8	2
Water	1	4	4	3	3	1	2	3	2	3	2.6	2.4	2.8
	TotGrowth	TotGrowth	TotGrowth	TotOverall	Boverall	Foverall							
D2	2	3	2.5	3	2.5	2	3.5	2.5	3	1.5	2.55	2.7	2.4
Daybreak	4	2	3	3	4	2	2.5	3.5	2.5	4	3.05	3.2	2.9
Marble/Granite	2.5	2	3.5	2	1.5	3.5	1.5	2	2.5	1.5	2.25	2.3	2.2
Water	1.5	3	2.5	2	2	2.5	2.5	3.5	2	3	2.45	2.1	2.8

Rankings from 1-4, ties allowable

(lower numbers indicates worse performance)

Appendix I.
Cost Estimates, Four Options for
Continuing the Study Two Years

Budget Option A, Four Cemeteri	es, Two Annual Fi	eld Trips	\$	
Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	360 hr	\$	29.05	\$10,458.00
Mary Striegel	80 hr	\$	59.50	\$ 4,760.00
Travel				
R/t Airfare	2 trips	\$	1,200.00	\$ 2,400.00
Per Deim	2 trips	\$	1,583.00	\$ 3,166.00
Mitchell Lab Biological analysis	128 samp	oles \$	5 250.00	\$32,000.00
				\$52,784.00

Budget Option B, Jefferson Ba	arracks & Alexandria	a, I WO FIE	ıa ırıps	
Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	280 hr	\$	29.05	\$ 8,134.00
Mary Striegel	80 hr	\$	59.50	\$ 4,760.00
Travel				
R/t Airfare	2 trips	\$	800.00	\$ 1,600.00
Per Deim	2 trips	\$	545.00	\$ 1,090.00
Mitchell Lab Biological analysis	s 64 sam	ples \$	250.00	\$ 16,000.00
				\$31,584.00

Budget Option C, Alexandria, Four	Field Trips, inc	ludes Bio	-Activity	/	
Salaries & Benefits	Quantity	Unit	Rate		Cost
Jason Church	120 hr	\$	29.05	\$	3,486.00
Mary Striegel	40 hr	\$	59.50	\$	2,380.00
Travel					
R/t Car Fare	4 trips	\$	50.00	\$	200.00
Per Deim	0 day	\$	-	\$	-
Mitchell Lab Biological analysis	32 samp	ples \$	250.00	\$	8,000.00
				\$	14,066.00

Budget Option D, Alexandria, Fo	ur Field Trips, Apլ	pearance	Only	
Salaries & Benefits	Quantity	Unit	Rate	Cost
Jason Church	120 hr	\$	29.05	\$ 3,486.00
Mary Striegel	40 hr	\$	59.50	\$ 2,380.00
Travel				
R/t Care Fare	4 trips	\$	50.00	\$ 200.00
Per Deim	day	\$	-	\$ -
Mitchell Lab Biological analysis	samp	oles		\$ -
				\$ 6,066.00

Section 3

Phase II: Chemical and Physical Testing for the Evaluation of Effects of Cleaners on Marble

Mary F. Striegel, Jason Church, Georgette Lang, and Lauren Vienne

National Center for Preservation Technology and Training 645 University Parkway Natchitoches, Louisiana, 771457

Introduction

The National Center for Preservation Technology and Training (NCPTT) compared the effectiveness of five commercially available cleaners for use on government issued headstones with funding from the National Cemetery Administration (NCA, an office of the U.S. Department of Veterans Affairs.)

The goals of the project were to:

- Evaluate a series of commercially available products in the field and in laboratory experiments.
- Test products that are User friendly, suitable for large-scale cleaning projects, environmentally friendly, and cost effective.
- Study the cleaning effectiveness, inhibition of re-growth, ease of use, and potential long-term damage to the stone.

The project was begun in 2004 and executed in three phases. It included both field and laboratory testing.

Phase one of the study focused on field trials undertaken in five national cemeteries distributed geographically and climatically across the country. Cemeteries included Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM.

Water and five commercially available cleaners, including D/2 Biological Solution, Daybreak cleaner, World Environmental Group Marble cleaner, H2Orange Grout Safe cleaner, and Kodak Photo-Flo were evaluated at each cemetery. Cleaners were applied to test patches on headstones carved from Colorado Yule marble and White Cherokee Georgia marble. Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations.

Based on the field trials, two cleaners were eliminated from further study. Kodak Photo-Flo was eliminated from further testing after six months due to changes in appearance and reoccurrence of biological activity. This product was a poor performer at controlling bacteria in both sunny and shady locations in all cemeteries. It also ranked the lowest of all cleaners in limiting biological activity overall. H2Orange Grout Safe Cleaner was eliminated because it left an undesirable surface appearance for a period of time after cleaning.

Phase two of the study focused on possible physical and chemical changes to the marble after treatment with one of three cleaners -- D/2 Biological Solution, Daybreak cleaner, and World Monument Group Marble and Granite cleaner. Evaluation of field samples and lab samples included microscopy, conductivity, colorimetry, profilometry, porosimetry, and artificial aging tests.

Phase three of the study will be reported on separately. It focused on microbiological studies in the laboratory to determine which of three biocides is most effective in the protection of marble against regrowth of microbial films.

Methods and Materials

One inch block samples were cut from each field test stone. Two stone types were examined – Colorado Yule marble and Georgia Cherokee marble. All samples were photographed prior to analyses.

Microscopy

We used a Leica MZ8 boom microscope with a total magnification range from 6.3X to 50X. All samples were examined with 25X magnification and were photographed using the microscopes' digital Spot camera attachment.

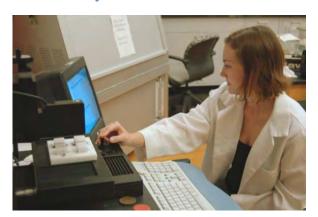
Conductivity

We used a Thermo Scientific Orion 4-Star Plus pH/Conductivity Portable Multiparameter Meter to measure conductivity of each solution. The meter has a conductivity accuracy of 0.01µS/cm.

Colorimetry

Color measurements were taken using the Konica Minolta CR-400 colorimeter and SpectraMagic NX Pro-USB version 1.52 software. Data was collected using a two degree observer setting and primary C illuminant.

Profilometry



Solarius LaserScan, a 3-D non-contact laser profilometer, was used to characterize stone sample surfaces. The instrument uses a class II diode laser (670 nm wavelength) and a 2 μ m spot size. The vertical resolution of this instrument is 0.1 μ m. The maximum vertical range is 1 mm. This range allows for the measurement of surface peaks and valleys

typically encountered on stone surfaces. The laser is scanned over an area of 31.07 mm (x-axis) by 23.02 mm (y-axis) at a scan speed of 5 mm/s and a resolution of 25 μ m. ¹ An estimated run time per sample is 111 minutes.

Porosimetry

Analysis of samples was contracted using mercury intrusion porosimetry (Porous Materials, Inc. Analytical Services Division, Ithaca, NY). Samples were analyzed using an automated mercury intrusion porosimeter (PMI model AMP-30-A-K-1). Results included Pore Volume, Pore Size Distribution, and Surface Area for a Colorado Yule marble control sample, a Colorado Yule marble sample cleaned with D2 biological solution, and a Colorado Yule marble sample cleaned with Daybreak.

Artificial Aging

All accelerated weathering studies used a Q- Panel Lab Products model QUV/ Spray Accelerated Weather Tester (weatherometer). This instrument uses panels of UVA-340 lamps to control a programmable cycle of light and dark. The bulbs irradiance level is calibrated to a constant level of 0.77 W/m2.

The Weatherometer was programmed for a continuing cycle of UV exposure for 4 hours at 60 degrees C followed by 4 hours of condensation at 50 degrees C. Note that this step was in the dark (no UV light) to mimic the natural cycle of night and day, and the temperature drop encouraged condensation from the surrounding humid air inside the Weatherometer. The water that condensed inside the Weatherometer initially comes from a lower holding pan that was supplied from a filtered water system that generated 18 megohm-cm purity of water. These cycles repeat for a total of 800 hours.

Three different trials:

- 1) spray cleaners on a 24 hour cycle, no rinse
- 2) Cleaned at the beginning of the QUV weathering, then spray cleaners on a 7 day cycle, rinse following cleaner
- 3) New formulation of D/2 tested with Daybreak, Cleaned at the beginning of the QUV weathering, then spray cleaners on a 7 day cycle, rinse following cleaner

¹ Other conditions include a row pitch of 85.95 and a column pitch of 88.33.

Results

Microscopy

Samples of Colorado Yule were dry cut from field stones that had been placed in next to headstones in the cemeteries during field trials. These stones had been cleaned once over the course of 18 months. Figure 1 shows images of possible efflorescence found on exposed surface of samples from the field as seen under the microscope.





Figure 1. These images were gathered from field samples placed in the cemetery during field trials.

The micrograph on the left shows possible salts from D/2 cleaning collecting on the surface of a Colorado Yule sample.

The micrograph on the right show possible salts from Daybreak within a pore of the stone. All micrographs were taken at 25X magnification.

Conductivity

Conductivity was used to determine if soluble salts were left on the field stones after cleaning. Samples were ground to a fine powder. A portion of the powder was weighed then soaked in a fixed volume of reagent grade water. The conductivity of each solution was measured in micro-siemens.

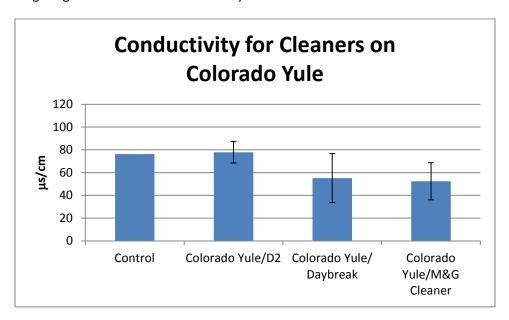


Figure 2. This graph shows the conductivity of Colorado Yule samples before and after cleaning with D2, Daybreak, or M&G cleaner. Little change is seen between the control sample and the cleaned samples.

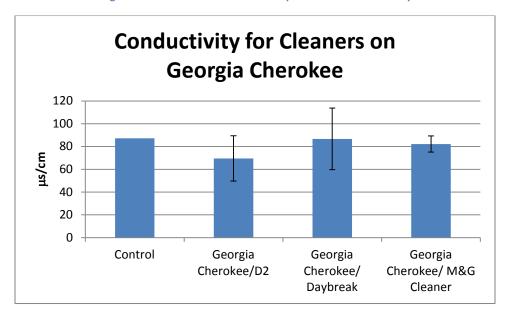


Figure 3. This graph shows the conductivity of Georgia Cherokee marble before and after cleaning with D2, Daybreak, and M&G Cleaner. Little change is seen between the control and the cleaned Georgia Cherokee samples.

Colorimetry

Color measurements were taken on randomly selected samples from the five field test sites to determine if discoloration was resulting from the use of the test cleaners. Three samples were selected for each cleaner: D/2, Daybreak, and Marble& Granite Cleaner.

Color Change in Colorado Yule Samples

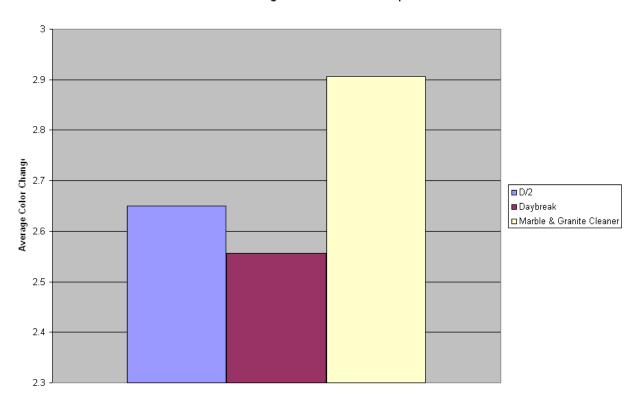


Figure 4. This plot shows the total color change, ΔE , for three Colorado Yule marble samples for each cleaner: D/2, Daybreak, and M&G cleaner. Samples were randomly chosen from field samples that had weathered outdoors. All values were less than 3.0 which means that they are not visual to the human eye.

Color Change in Cherokee Georgia Samples

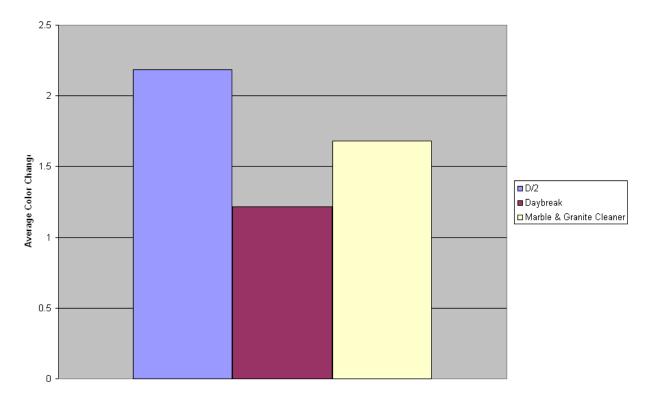


Figure 5. This plot shows the total color change, ΔE , for three Georgia Cherokee marble samples for each cleaner: D/2, Daybreak, and M&G cleaner. Samples were randomly chosen from field samples that had weathered outdoors. All values were less than 3.0 which means that they are not visual to the human eye.

Profilometry

Laser profilometry was used to study the un-cleaned and cleaned samples to determine if significant surface texture changes could be found. Surface roughness parameters included average surface roughness (Sa), core roughness (Sk), roughness of peaks (Spk) and roughness of valleys (Svk).

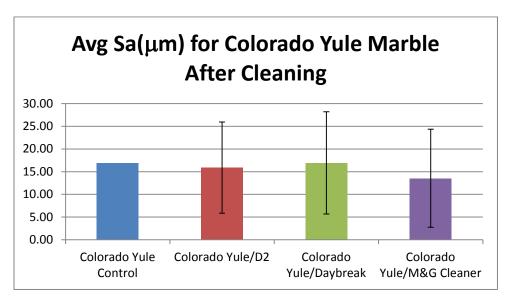


Figure 6. The overall average surface roughness for Colorado Yule Marble samples before and after cleaning. All cleaned samples show similar overall roughness when compared to the control sample, with Sa values between 13-16 μ m.

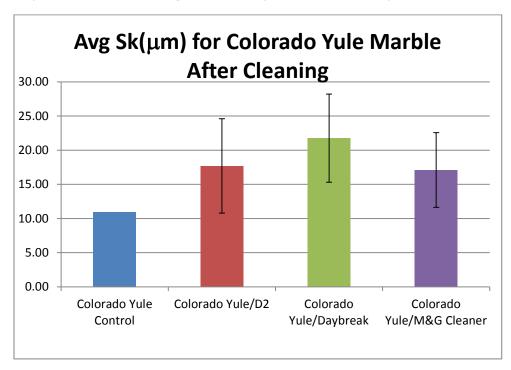


Figure 7. The average core roughness, Sk, increases upon cleaning for all cleaners used. Daybreak results in the greatest average core roughness.

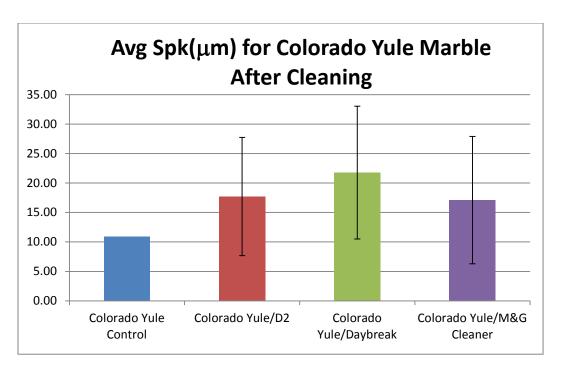


Figure 8. The average peak roughness of replicate samples is shown for Colorado Yule marble before and after cleaning with D2, Daybreak, and M&G cleaner. All cleaned peaks are rougher than the control sample. Samples cleaned with Daybreak indicate the roughest average surfaces.

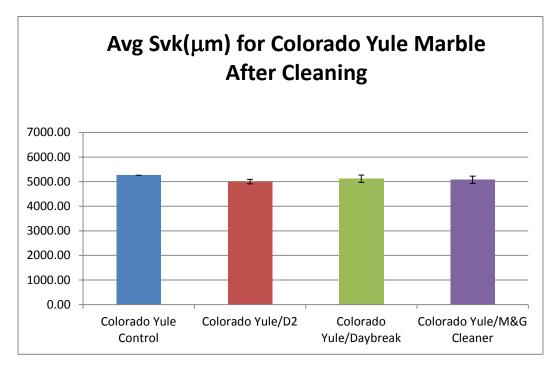


Figure 9. The average valley roughness of replicate samples is shown for Colorado Yule marble before and after cleaning with D2, Daybreak, and M&G cleaner. All roughness of the valleys remain similar.

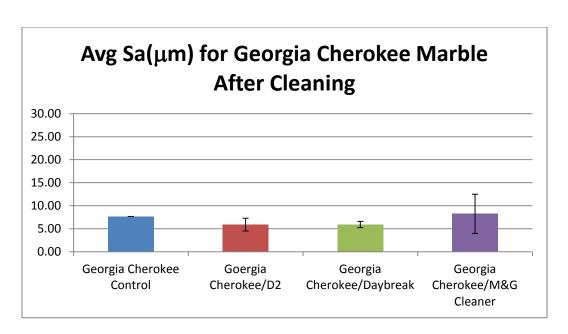


Figure 10. The overall average surface roughness for Georgia Cherokee Marble samples before and after cleaning. All cleaned samples show similar overall roughness when compared to the control sample, with Sa values between 6-8 µm.

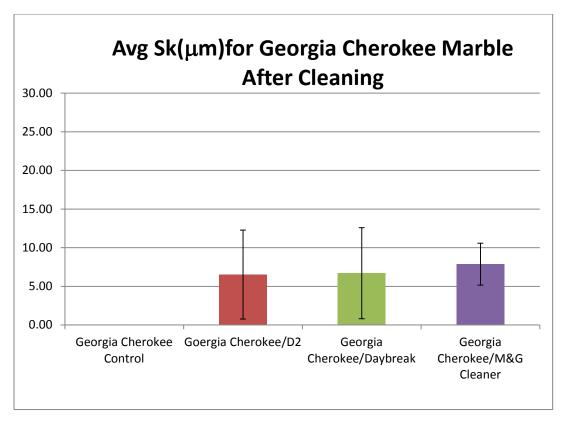


Figure 11. The core roughness value, Sk, for Georgia Cherokee marble before and after cleaning. The core roughness for the control sample is 0.00, while the cleaned samples are similar ranging between 6.53 for D2 cleaned samples to 7.87 for M&G cleaned samples.

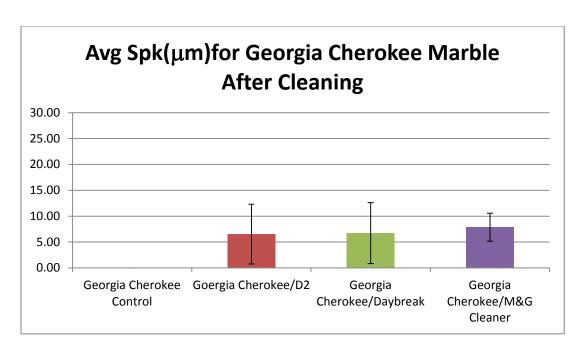


Figure 12. The peak roughness values, Spk, for Georgia Cherokee marble before and after cleaning. The control sample showed no roughness, while the cleaned samples are similar with only slight changes.

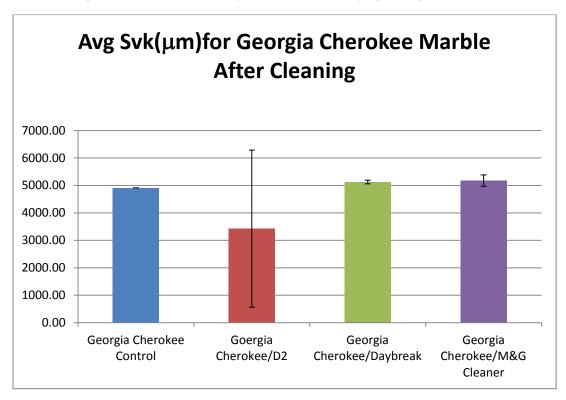


Figure 13. The average Valley Roughness, Svk, is plotted for Georgia Cherokee marble before and after cleaning. All samples show similar Svk values. The greatest variability was seen in the samples cleaned with D2.

Porosimetry

Mercury porosimetry was undertaken to determine if cleaning the samples were leading to changes in the pore structure of the stone. Three samples of Colorado Yule were analyzed: an untreated control sample, a sample cleaned with D/2, and a sample cleaned with Daybreak.

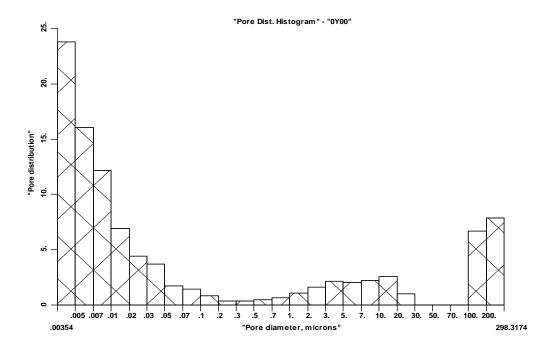


Figure 14. This graph shows the pore distribution for an untreated control sample of Colorado Yule marble as determined by mercury porosimetry. From left to right, micro-pores are less than 2 nm, meso-pores are from 2 nm to 50 nm range, and macro-pores are greater than 50 nm.

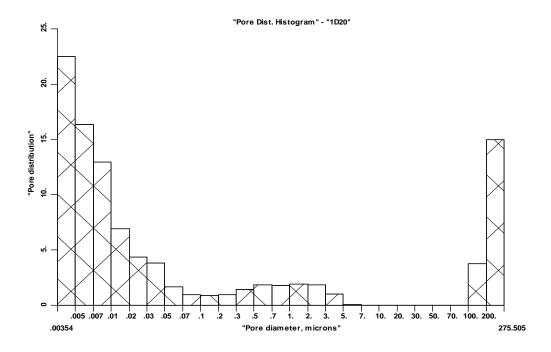


Figure 15. Sample 1D20 is a D2 treated sample of Colorado Yule marble from Jefferson Barracks National Cemetery. While mirco-pores show little change from the control samples, meso-pore distribution has decreased and more macro pores can be seen above the 200 nm range.

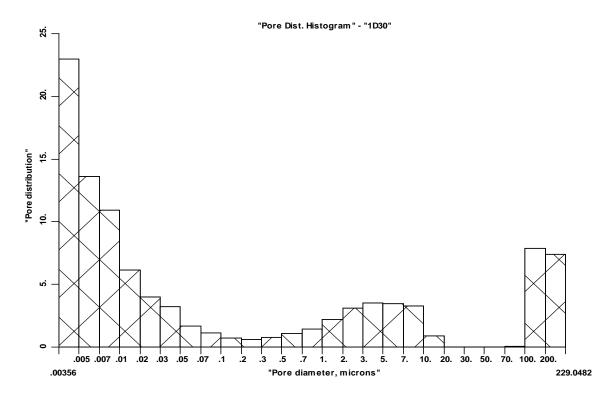


Figure 16. Sample 1D30 is a Colorado Yule sample treated with Daybreak and exposed in Jefferson Barracks National Cemetery during field trials. Increases are seen in meso-pore size distribution between 0.5 nm and 20 nm.

Artificial Aging

Experiment 1: 33 days, cleaned daily

Artificial aging experiments were undertaken to gather more information about possible effects of the test cleaners on Colorado Yule and Georgia Cherokee marbles. A series of samples were cored from the study marble. Samples were photographed and documented by laser profilometry. Next samples were cleaned with either water, D/2, Daybreak, Kodak Photo-flo, Granite & Marble Cleaner, or H2Orange2. Samples were mounted onto Teflon holders and placed inside the QUV weatherometer. Samples were randomized and rotated on a daily basis to possible systematic errors.





Figure 17. On right samples are cored from Colorado Yule Marble. On left samples are placed into QUV weathering tester.

Cleaners were applied once a day before rotating the samples. Samples were removed from the QUV, by groups, a spray guard was placed so that only the sample in question would receive the cleaner. One pump of cleaner was sprayed on each surface. The testing continued for 33 days. This study represented the worst case scenario, since cleaners were applied frequently and not rinsed.

After 33 days, salt crystal growth was seen on samples cleaned with Daybreak and D/2. No other efflorescence was seen with the other cleaners.

Experiment 2: 33 Days, cleaned every 7 days and followed with a rinse

A second trial of artificial weathering was conducted under less harsh conditions more similar to actual treatments in the field. New samples of Colorado Yule and Georgia Marble were cored and sliced into coins. With this trial, samples were cleaned with either D/2 or Daybreak. Samples were rotated on a weekly basis. They were cleaned in groups suing a shield guard to prevent cross contamination. One pump of cleaner was sprayed on each surface and allowed to dwell for the manufacturer's recommended time. Each sample was then rinsed with clean water. The testing continued for 33 days.

After 33 days, large salt crystals were observed on samples cleaned with Daybreak. Additionally, a fine powdery salt formation was found on samples cleaned with D/2. Both alteration products were sent to Columbia University for identification by X-ray Diffraction. Identification of the crystalline materials by XRD was inconclusive.

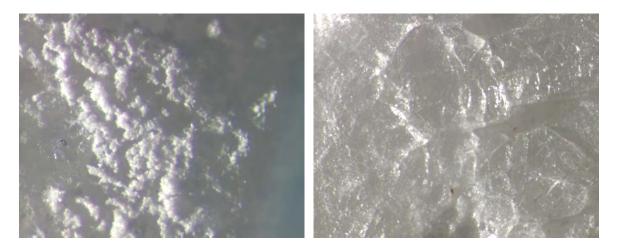


Figure 18. Surfaces of Colorado Yule marble samples were observed under 100X magnification. In the left image: a fine, powdery salt efflorescence was observed on the sample cleaned with D/2 and artificially weathered for 33 days. In the image on the right, large salt crystals grow from the surface of the sample cleaned with Daybreak and artificially weathered for 33 days.

Experiment 3: New Formulation, 33 Days, cleaned every 7 days and followed with a rinse

Based on NCPTT's results, the manufacturer of D/2 Biological solution was contacted. A representative² of the company indicated that a buffering agent used to adjust the D/2 cleaner may be leaving trace salts on the samples. Based on this discussion, the manufacturer reformulated the cleaner and removed the buffering solution. They provided NCPTT with the newly formulated cleaning for laboratory testing.

The third trail consisted of Colorado Yule and Georgia samples cleaned with reformulated D/2 and Daybreak. Testing was performed under conditions identical to Experiment 2, above. Upon conclusion of the exposure, no salts were observed on the reformulated D/2 samples, while Daybreak treated samples continued to grow large salt crystals.

-

² Private Communication, between Jason Church and Ted Kinnari, 2009.

Discussion

NCPTT researchers used a variety of techniques to investigate changes from un-cleaned control samples to cleaned stones. Two types of stone samples were studied. The first type of stones were cut and placed in five locations along site headstones in the study. These cut stones were treated with five cleaners and water in the field. They were exposed for 18 months after cleaning, before being removed and shipped to the lab for testing..

Upon removal of the field test stones, most testing was conducted by Lauren Vienne, a graduate student at the University of Texas Austin. Her work was undertaken at NCPTT during a summer internship. She investigated changes to the stone using microscopy, conductivity, colorimetry, profilometry, and porosimetry. Additionally, she looked at the samples using Scanning Electron Microscopy, the results of which were inconclusive.

Initial examination of the field samples of Colorado Yule showed possible salt efflorescence seen on cleaning areas treated with D/2 or Daybreak, as shown in Figure 1. These results could not be confirmed with Scanning electron microscopy.

To test the hypothesis that residual salts might be found on the field stones, samples were dry cut from the stone and analyzed using conductivity. If salts were present, then one would expect an increase in conductivity from ions dissolved in solution. No significant differences were seen between D/2, Daybreak, or Marble & Granite Cleaner on either Colorado Yule or Georgia Cherokee marble samples. One possibility is that no salts were formed on the surface based on only one cleaning. Alternately, it is possible there were not enough residues on the surface to be detectable by the methods employed in this study.

Possible changes to the surface color of Colorado Yule and Georgia Cherokee marble upon natural weathering outdoors was investigated using color measurement in CIE color space. A change in total color, ΔE , was calculated for stones selected from the field. Three stones were selected for each cleaner and marble type. These results are shown in Figure 2 for Colorado Yule Marble and Figure 3 for Georgia Cherokee Marble. No significant differences were observed between the cleaners on each stone type. No yellowing or change of color took place over the 18 month exposure period. Residues left on the surface, if any, were UV stable based on this test.

These results indicate that that no detectable levels of residues were left on the surface of stones after only one cleaning. Maintenance practices that require annual or even more frequent cleaning may lead to very different results. The practice of leaving a cleaner on the surface without rinsing was not tested in this study.

Next, NCPTT investigated possible physical changes to marble surfaces upon cleaning with D/2, Daybreak, or WEG Marble & Granite Cleaner. Did surface texture change after cleaning? Change in surface texture may lead to greater re-soiling by both dirt and microbes because of a greater surface area. This question was studied using laser profilometry. The average surface roughness (Sa), the average core roughness (Sk), the average peak roughness (SpK), and the average valley roughness (SvK)

were calculated for three test cleaners on each marble. Results of the cleaned surfaces were compared with a control sample in each case.

The average surface roughness, (Sa), for Colorado Yule marble samples, shown in Figure 6, is always greater than the average surface roughness of Georgia Cherokee marble samples, shown in Figure 10. Colorado Yule samples were almost three times rougher than the Georgia Cherokee samples. This is likely due to the nature of the stone. Colorado Yule marble is a fine grained stone which may be less dense than the larger grained Georgia Cherokee stone. Thus Colorado Yule marble may naturally be more susceptible to soiling than Georgia Cherokee marble.

Based on average surface roughness, no significant changes were seen between any of the cleaners applied. Sa values are considered a general roughness parameter and may not accurately characterize fine changes to a stone surface.

Researchⁱ undertaken by ElizaBeth Bede Guin, indicates that surface texture parameters such as the average core roughness (Sk), the average peak roughness (SpK), and the average valley roughness (SvK) help to differentiate stones. The fore mentioned parameters are defined in Table 1, below. The values are based on calculations from a curve known as the Abbot Firestone Curve, an example of which is shown in Figure 19.

Table 1. Definitions of surface metrology parameters used to evaluate surface changes on Colorado Yule and Georgia Cherokee marbles before and after cleaning with test cleaners.

Parameter	Туре	Name	Definition					
Sk	3D Roughness of		Calculated as the height difference between the					
	Functional	the core	intersection points of the found least mean square line.					
	Parameter		The determination of the mean line uses Gaussian					
			filtering defined in ISO 13565-1. Once the mean line is					
			determined, an Abbott Firestone curve or cumulative					
			probability distribution is calculated. The Sk value is					
			determined from the Abbott Firestone curve using a 40%					
			window.					
Spk	3D	Roughness	The mean height of the peaks protruding from the					
	Functional	Depth of	roughness core profile. Calculated as the height of the					
	Parameter	Peaks	upper left triangle on the Abbot Firestone curve					
Svk	3D	Roughness	The mean depth of valleys protruding from the roughness					
	Functional	Depth of	core profile. Calculated as the depth of the lower right					
	Parameter	Valleys	triangle on the Abbott Firestone curve.					

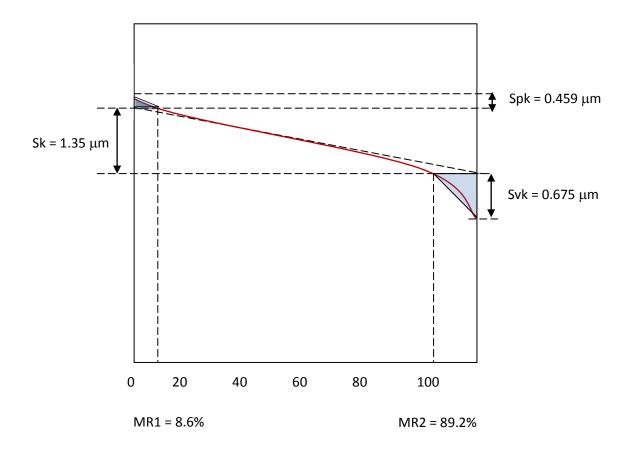


Figure 19. Diagram illustrating the core roughness parameter, Sk, as calculated from the Abbott Firestone Curve. Also shown are the roughness depth of the peaks, Spk, the roughness depth of the valleys, Svk, and the percentage of materials at the beginning and end of the 40% window, Mr1 and Mr2.

Was the pour size and distribution affected by cleaning?

The second type of stone samples was examined solely in the lab, using artificial weathering as a means to evaluate possible long-term changes in short-term exposure. Three trials were developed and undertaken by Georgette Lang, an undergraduate student at Centenary College and a summer intern, or by Jason Church, NCPTT's materials conservator.

Conclusions

ⁱ Bede, ElizaBeth A., "The Surface Morphology of Limestone and its Effect on SO2 Deposition" (Ph.D. diss. University of Delaware, 2001).

Section 4

A report on an evaluation of antimicrobial activity of three biocides on marble

To

Mary Striegel

NCPTT, National Park Service

Investigators:

Ralph Mitchell, Gordon McKay Professor of Applied Biology Archana Vasanthakumar, Postdoctoral Fellow Conor O'Herin, Undergraduate researcher Alice DeAraujo, Research assistant

School of Engineering and Applied Sciences Harvard University

December 2010



Objectives

In a laboratory study, we used disks of Colorado and Georgia marble to test the ability of three biocides, DaybreakTM, D/2 and WEG marble cleaner, to resist the challenge by a high concentration of microorganisms. This report describes the results after 188 days of testing in a high humidity environment at a temperature of 72°F.

Conclusions

We tested the ability of three biocides, D/2, DaybreakTM and WEG marble cleaner, to control microbial growth on Colorado and Georgia marble under accelerated laboratory conditions. The temperature was maintained at 72°F and the humidity at 80%. Marble disks were inoculated with the microorganisms in a growth medium and treated with the biocides. We used mold (fungi) growth as an indicator. After 188 days, the WEG cleaner had failed to control fungal populations on Colorado marble but not on Georgia marble, indicating that it is not a good candidate for use in the field. D/2 and DaybreakTM continued to keep fungal populations low on both types of marble. We conclude that the WEG cleaner was not as effective as D/2 and DaybreakTM in preventing fungal growth, after 188 days of testing. Both D/2 and Daybreak were highly effective in controlling mold growth.

This is the final report of this project. However, we will continue to monitor the marble disks.

We plan to determine how long the two biocides remain active and compare the biocidal activity on the Colorado and Georgia marble.

Methods

Experimental set up

Two types of marble, Colorado and Georgia, were used in this study. Marble disks were heatsterilized before the experiment was set up. A fungus, *Aspergillus niger*, was chosen for inoculation on the marble surface, as our indicator of biocidal activity.

Fifty microliters of a microbiological growth medium were placed on the surface of each disk. Once the media air dried, 20 µl of fungal suspension was added to each disk. Disks inoculated with fungi were placed in plant growth trays (Figure 1), which in turn were placed on a flat tray containing water. Clear plastic domes were used to cover the plant growth trays, thereby helping to keep the humidity high. All inoculated disks were maintained at high humidity and at room temperature (ca. 72°F) for a total of 7 weeks before treatment with biocides.

The day before biocide treatment, three disks for each marble type were set aside as controls.

These were marked positive controls and were not treated with biocides.



Figure 1: Experimental set-up showing marble disks arrayed in a plant growth tray.

Sampling microbial populations

Disks were swabbed in order to obtain a baseline estimate of the concentration of microorganisms before cleaning. Sterile swabs were used to sample the surface of inoculated marble disks. Swab samples were then dipped into 0.5 ml sterile water so that microorganisms could be transferred to the water. Fungi were quantified using a rapid fluorimetric assay developed in our laboratory. A high concentration of microorganisms was detected before biocidal treatment.

Biocides

Three biocides, D/2, Daybreak[™] and World Environment Group's (WEG) marble cleaner, were tested. D/2 is an architectural biocide. Daybreak[™] is a mildew stain remover. The WEG marble cleaner is a soy-based, environmentally friendly cleaner. Appropriate concentrations of the three biocides were made up in the sprayers provided. Inoculated disks were treated in the laminar flow hood with a quick 2-second spray onto the top surface (the inoculated surface) of each disk. Two rounds of spraying were performed with 1 min between sprays. About 3 minutes after the 2nd round of spraying, paper towels were used to mop up excess liquid on the surface of disks. This process was repeated for each cleaner. Once the disks were "dry", they were transferred back to the plant growth trays in order to maintain high humidity. They were then incubated at room temperature. The relative humidity was ca. 80%. Representative disks were sampled on day 1 (the day after biocide treatment) as well as at the sampling times shown in figures 2 and 3.

Results

We tested the effectiveness of three biocides, D/2, Daybreak[™] and WEG marble cleaner in preventing growth of microorganisms on Colorado and Georgia marble. We sampled the disks for microbial growth at the following times, after spraying with the biocides:, Day 1, Day 34,

Day 41, Day 90, Day 127 and Day 188. The effects of the biocides in the control of microbial growth on Colorado marble are shown in Figure 2. The results for Georgia marble are shown in Figure 3.

Soon after biocide treatment (Day 1 sampling time), almost no fungi were detected on the treated stone disks. The biocides had reduced the microbial population to nearly zero. In contrast, the microbes on the untreated disks continued to grow well. All three biocides controlled the biological activity equally. At days 34 and 41, higher fungal populations, relative to Day 1, were detected. The fungal population slowly increased with time, as shown in Figures 2 and 3, though it was still very low compared to initial populations. After 127 days, there was no apparent difference among the activity of the three biocides. At 188 days post biocide treatment, fungal populations had increased considerably on Colorado marble disks treated with WEG cleaner. However, the populations on Georgia marble treated with the same biocide did not increase at the same rate. Fungal growth on marble treated with D/2 and DaybreakTM increased but were still low compared to initial populations.

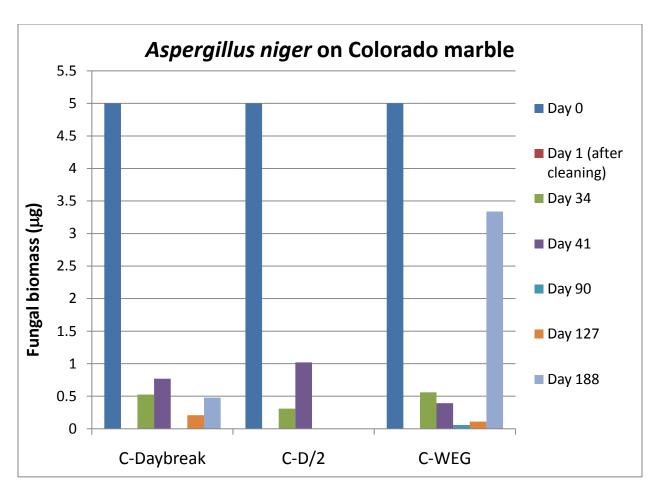


Figure 2: Effects of the three biocides, DaybreakTM, D/2 and WEG marble cleaner on fungal growth on Colorado marble

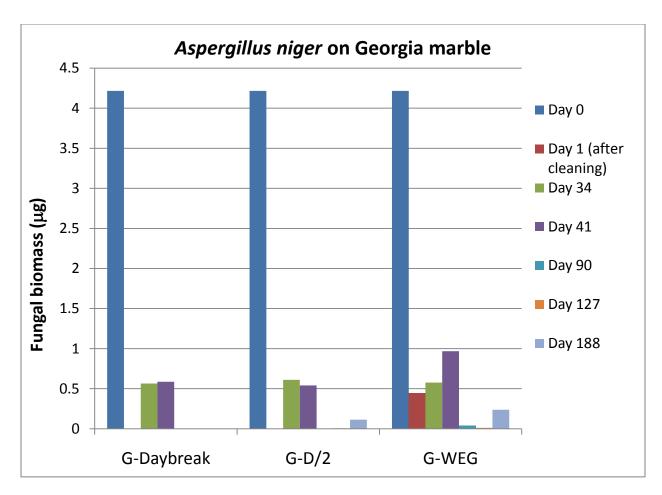


Figure 3: Effects of the three biocides, DaybreakTM, D/2 and WEG marble cleaner on fungal growth on Georgia marble

The results of our laboratory investigation indicate that both D/2 and Daybreak are good candidates for long term field studies. The failure of WEG in our tests after 188 days suggests that, at least with Colorado marble, it may fail in the field after prolonged exposure.

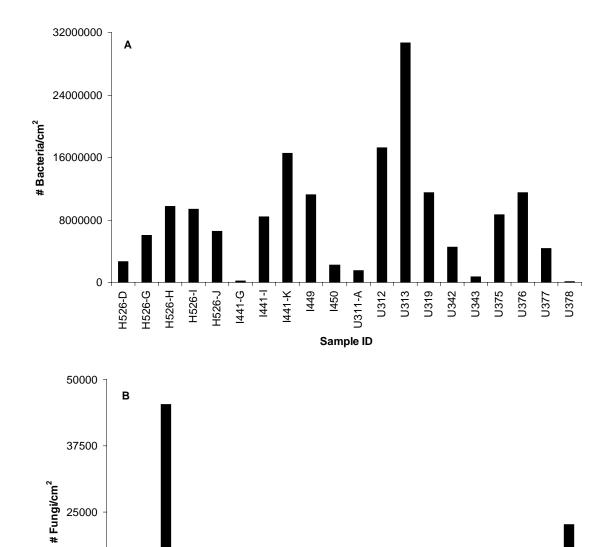


Figure 1-5. Numbers of bacteria (A) and fungi (B) in samples from Santa Fe National Cemetery.

1441-K

1449

U313 U342 U343 U375 U376

U311-A U312

Sample ID

U377

1441-G

1441-

12500

H526-G

H526-H

H526-I H526-J

Section 5



Best Practice Recommendations for Cleaning Government Issued Headstones¹

This document was developed as general guidance for the cleaning of government issued headstones based on research undertaken by the National Park Service National Center for Preservation Technology and Training and funded by the Department of Veterans Affairs National Cemetery Administration. Recommendations are intended to be used by cemetery directors, operations staff, foremen, maintenance staff, contractors and headquarters staff. The document focuses on general cleaning and regular maintenance of marble headstones that are soiled from dirt and biological growth. Recommendations do not address cleaning needs from unusual events such as removal of road tar, mower scars, vandalism, or other accidental damage. Cleaning recommendations for other stone types such as granite, sandstone, or limestone are not presented here.

One of the critical components of maintaining the appearance of a national cemetery is the cleaning of headstones. Many of the more than 3 million gravesites in 131 national cemeteries are historic headstones and markers which should be protected and treasured. Also, today's new headstone will be tomorrow's historic grave marker.

Headstone cleaning must take into consideration the operational standards set forth by the National Cemetery Administration. [1] The following standards are among those designated for headstones:

- Headstones, markers, and niche covers are clean, free of debris and objectionable accumulations.
- Headstones, markers, and niche covers are not damaged by cemetery operations (e.g., interment, grounds maintenance, headstone, marker, niche cover, maintenance, and facility maintenance operations).

Maintenance practices must have an eye toward the future. Many cleaning methods may be able to remove soiling from headstones. Some will be more effective than others. But the long-term effects must also be considered. Anyone developing a cleaning method must look at the soiling agent to be removed, the potential threats caused by the soiling, and the possible unintended results of cleaning.

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¹ This document, released for distribution on May 23, 2011, is part of a forthcoming report of research undertaken by the National Park Service's National Center for Preservation Technology and Training for the Department of Veterans Affairs National Cemetery Administration.

Soiling Agents or Accumulations

Soiling agents are accumulations on stone that alter the appearance of the stone and may cause additional damage. Different soiling agents may respond better to a particular cleaning method. Soiling agents include:

- **Dirt**, including soil and mud, often arises from transferring the topsoil to headstone surface. Dirt can lead to dark staining on the surface or an overall dingy appearance. Dirt can penetrate into the pores of the stone and be difficult to remove. Minerals containing iron can leach into the marble surface and leave rust colored stains behind. If the headstone has sunk into the ground over time, then is raised and realigned, a distinct line of soiling can be seen. Dirt can retain moisture after rainfall and lead to the growth of mold or mildew on the stone surface.
- Air pollution, including particles from vehicle exhaust, can deposit on the surface of
 marble. Nearby factories or industrial activities can generate pollutants that can change
 the appearance of the stone or chemically interact with the stone over time. For
 example, sulfur dioxide produced through manufacturing processes and vehicle exhaust
 can interact with marble surfaces to cause gypsum crusts. These crusts can capture soil
 and pollution particles to create rough, gray surfaces.[2]
- Biological organisms, such as bacteria, mold, mildew, algae, mosses, or lichen can adhere to the headstone and result in appearance changes. Microorganisms are capable of establishing a biofilm on the surface of the stone. Biofilms include proteins and sugars that are hard to remove through standard cleaning practices and provide food for regrowth of organisms.[3] Bacteria can consume air pollutants and produce acids that can attack the stone. Fungi can penetrate the pore system of stone and carry bacteria further into the stone.[4]
- **Bird droppings** or other animal secretions can stain the stone. Depending on the animal's diet, the stains may be difficult to remove. Urine seeps into porous materials and with time produces yellow stains.
- **Plant or tree sap** is a sticky substance that drips from overhanging trees. The material may contain resins that are not easily dissolved in water. The sugars in the sap may attract insects or provide food for molds and mildews. Shrubs have falling berries that can stain surfaces.

Other threats to headstones

- **Salt damage** can cause disintegration of a stone surface. The presence of salts within the stone, in the grounds surrounding the stone, in irrigation water, in some herbicides, and in some cleaners, can migrate through the stone's porous network and cause damage. Salts are dissolved and transported by water. They can recrystallize and exert pressures in the pores that may exceed the strength of the stone.[5, 6] Thus, do not use cleaners that leave behind salts to clean marble headstones.
- Freeze thaw cycles can increase stone weathering. Water can enter into openings, cracks, and pores of stone. If freezing temperatures exist, the water can freeze and expand. With many freeze thaw cycles, water can damage stone.[7] Since most cleaning efforts require saturating the stone with water or liquids, do not clean headstones during freezing temperatures or when a freeze is expected within 48 hours of the cleaning.
- *Improper cleaning* can stain the surface or accelerate stone deterioration. Well-meaning but ill-informed custodians of cemetery headstones do damage through poor selection of cleaning methods. This would include use of power-washing equipment too close to the stone, not rinsing after application of cleaner, and using products in a greater strength than the manufacturer recommends.

Important factors to consider

• Use the gentlest, least invasive method

Select cleaning methods and materials that, to the best of your knowledge, do not affect the headstone. Chemicals and physical treatments should be undertaken using the gentlest means possible to insure the longevity of the headstone and to minimize the need to replace the stone.

• Do no harm to the stone

Do no harm to the headstone during its care or the care of the cemetery. A headstone is placed on a soldier's grave as a marker to identify burial site, but serves other roles as well. It is intended to honor the deceased and thus should be treated with respect. Over time the headstone takes on meaning to the loved ones who visit. By its very nature, it possesses added value and association to the veteran's service.

• Consider long-term effects

Recognize that cleaning efforts are part of a continuum of cleaning that will be applied to the headstone. All efforts to clean headstones affect the surface in ways that are not always obvious. Marble is made up of interlocking grains of carbonate mineral which is bound together in a network that includes varying amounts of pores. When the surfaces are cleaned, some of the grains can be loosened and lost. Sometimes the mineral binder that holds the stone together can be affected. Over time and many cleaning campaigns, the surface can be altered noticeably and result in a sugaring appearance. Some marble is more prone to this type of deterioration than others. For example, Colorado Yule marble is more affected by cleaning than Cherokee White marble from Georgia.

• Don't remove the original surface

The original surface may be polished and smooth. The inscriptions are generally carved into the headstone. If the original surface is altered, the way the headstone subsequently weathers may be changed. As the surface roughens, it will soil more easily. The inscriptions can be eroded away, making the headstone harder to read. Never aggressively scrub the surface, or use wire brushes or mechanical methods such as sanders or grinders to clean the surface. See also –mechanical cleaning: power tools, below.

Minimize cleaning impacts

Minimize the number of times a headstone is cleaned in its lifetime. While a cyclic maintenance plan is needed to maintain the appearance of the headstone, over-cleaning should be avoided. If possible, historic headstones should not be cleaned more frequently than once a year.

• Test cleaner first

ALWAYS TEST the cleaner for suitability and results before overall cleaning. Conduct the test using the recommended application procedures. Let test area dry thoroughly before inspection. When using a biocidal cleaner, it may take several days before the full cleaning effect is realized. When practical, allow two or more weeks for biological soiling to disappear.

Consider Environmental Conditions

Environmental conditions may dictate the frequency of cleaning. For example, headstones that are located in shady and damp areas under trees may need to be cleaned more frequently than headstones in sunny areas.

Cleaning techniques known to damage stone

• Bleach or bleach-like products

Household bleach or other oxidizing cleaners, such as Daybreak cleaner or HTH Shock 'N Swim pool treatment may chemically react with the stone surface and leave soluble salts in the pores of the stone which will lead to decay. Check the label of the cleaner or the Materials Safety Data Sheet (MSDS) for active cleaning ingredients. If the products contain sodium hypochlorite (NaClO), sodium perborate, sodium percarbonate, sodium persulfate, tetrasodium pyrophosphate, calcium hypochlorite or urea peroxide, do not use them for cleaning the headstone. For example, Daybreak cleaner contains 14% sodium hypochlorite and is not recommended.

• Strong acids or bases

Strong acids, including muriatic acid, hydrochloric acid, or others are too harsh and will dissolve the stone surface. Because they are corrosive, they can also be hazardous to workers. Strong bases, such as concentrated ammonia, sodium hydroxide, calcium hydroxide, potassium hydroxide, or others may be aggressive on the surface of the stone and may be hazardous to workers.

Mechanical cleaning: Power tools

Harsh mechanical devices such as sand blasting, or power tools such as sanders or drills equipped with a wire brush remove the original material of the grave marker.

• Mechanical cleaning: High-pressure washing

Pressure washing systems are mechanical sprayers that use water under high pressures to clean surfaces. Commercially available pressure washers operate at pressures between 750 psi and 30,000 psi that will damage marble headstones. This technique can cut into and mar the surface of the stone. The appropriate distance and pressure needed to properly clean an individual headstone is generally about 12 inches with a pressure of 500 psi or less. Some stones may not be able to tolerate these conditions depending on their condition. A test patch in a small unobtrusive area on the headstone is recommended prior to cleaning.

Cleaning methodology

A cleaning regimen for headstones should be based on environmental considerations such as humidity, biological growth rates, tree cover and vegetation, precipitation and other factors that influence the frequency of cleaning necessary to maintain an appropriate appearance.

• Choosing the cleaner

Cleaning should be undertaken with the mildest, least-abrasive method. Improper cleaning can lead to accelerated deterioration or loss of original materials. Always begin by reviewing the Materials Data Safety Sheet (MSDS) for any chemical product to be used. The MSDS may be found by searching online or by contacting the manufacturer or distributor. The MSDS contains important chemical information and necessary safety precautions needed for use of the product.

Make sure to note the manufacturer's application recommendations. The two most important features to note are the dilution ratio and the dwell time. If the manufacturer recommends diluting the cleaner, use the recommended dilution ratio. A small amount of the cleaner should be added to water to create the required ratio. Using the cleaner in a more concentrated form may increase the risk of damage to the headstone. The dwell time is the amount of time that the cleaner is left on the surface of the stone before scrubbing and rinsing the stone. The dwell time varies depending on the cleaner.

Biocidal cleaners are available for use on stones that have biological growth, such as algae, mildew, moss, and lichen. Most biocidal additives also help to keep biological from returning to the stone for an extended period of time. Recommended biocidal cleaners include D/2 Biological Solution manufactured by Sunshine Makers, Enviro Klean® BioWash®, or other cleaners that contain quaternary ammonium compounds. Consult with the product manufacturer to determine if the biocidal cleaner contains buffers that may leave salts behind on the stone. Follow directions as specified by the biocide manufacturer, making sure to rinse thoroughly. It is important to know that marble cleaned with biocides should continue to lighten over the next few days. The advantage of a biocidal cleaner is that it helps remove a wide range of soiling including

² Exclusively distributed by Cathedral Stone® Products, Inc., 7266 Park Circle Drive, Hanover, MD 21076, Telephone: 410-782-9150, Fax: 410-782-9155.

³ Manufactured and distributed by PROSOCO, Inc., 3741 Greenway Circle, Lawrence, KS 66046. Telephone: 800-255-4255; Fax: 785-830-9797. E-mail: CustomerCare@prosoco.com.

biological growth. The disadvantage is that the cleaners are more expensive than other products on the market.

• Equipment needed

Personal Protective Equipment

While no special equipment is required under normal use, gloves and eye protection are recommended. Avoid eye contact where splashing of the cleaner may occur, such as during spray applications. Wash hands thoroughly after handling any cleaner and before eating, drinking or smoking.

Brushes

Soft bristle brushes are required when cleaning stones. They can have natural or synthetic bristles. Vegetable brushes or soft grooming brushes for large animals are a few that can be found in chain or farm supply stores. All rough or metal edges must be covered with tape to reduce the chance of scratching the stone.

Hand or Backpack Sprayers

A variety of hand-pump sprayers can be used for cleaning headstones. Make sure that the sprayer is dedicated to the cleaners to be used and not used for other functions like applying pesticides. Backpack sprayers are useful when cleaning a large number of headstones typical in the national cemeteries. These consist of a holding tank, hose, and wand with adjustable nozzle. The sprayers generally operate in a 15-80 psi pressure range.

Clean Water

One of the most important things to locate in the cemetery is the nearest source of water. It takes a lot of water to properly clean stone. If the cemetery does not have clean running water then it is important to bring barreled or bucketed water to the site.

• Pre-wetting the stone

Soak the stone liberally with water before applying the cleaner with a hand or backpack sprayer. Stone is a very porous material and will absorb the cleaner. By soaking it beforehand, the cleaner will stay on the surface of the stone and minimize penetration of the cleaner in to the stone. This action minimizes potential adverse effects by the cleaner, such as salt crystallization in the pores of the stone. It makes it easier to rinse the cleaner from the stone surface.

• Applying the cleaner

Always keep the stone wet during cleaning and thoroughly rinse afterwards. Do not allow the cleaner to dry on the stone. Apply the cleaner according to the manufacturer's recommendations. Changes to the dilution or dwell time are considered "off-label" and the effectiveness of the cleaning method cannot be guaranteed. Evenly apply the cleaner with a sprayer to saturate the surface.

• Agitating the surface

Agitate the surface gently in a circular motion using a soft bristle brush. Work in small areas, starting from the bottom and moving toward the top of the headstone. Agitation will loosen soiling from the surface of the stone.

• Rinsing the stone

Remember to rinse after cleaning each area and to thoroughly rinse the stone at the end to make sure that no cleaner is left behind.

A typical cleaning regime may include a three-person team. The first person thoroughly wets the stone with clean water using a hose or a portable backpack sprayer. A second person sprays the stone surface with the biocidal cleaner. After the appropriate dwell time, a third person gently agitates the cleaner on the stone surface with a soft bristle brush, then rinses the stone with clean tap water.

Glossary of Terms

Ionic cleaner: A substance that aids in the removal of dirt and serves as an emulsifier by bridging between water and oil. The substance is a long chain chemical that has a charge on one terminal.

Non-ionic cleaner: A substance that is similar to an ionic cleaner, except that it does not have a charge.

Surfactant: A compound that is a surface active agent. It reduces the surface tension between liquids that do not normally mix together. It aids in the cleaning of a surface.

Biocide: A chemical capable of killing living organisms.

Pressure washer: a mechanical sprayer that uses high-pressure water to clean and remove dirt and other accretions from surfaces and objects.

Dilution ratio: reduction of the concentration of a chemical by mixing with water or another solvent by a specific portion. A useful reference chart for specific dilution ratios can be found at http://www.tomorrowchemicals.com/files/Dilution Ratios TC.pdf.

Dwell time: The time a cleaner remains on the surface of a stone before agitation or rinsing.

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Section 6

Final Administrative Summary:

Comparative Evaluation of Commercially Available Cleaners for Government Issued Headstones Research conducted at the National Park Service, National Center for Preservation Technology and Training

For the Department of Veterans Affairs, National Cemetery Administration.

Submitted October 17, 2011 Mary F. Striegel

Time Period of Research: Three Phases, 2004-2011

Funding:

FY 2004 IA V101(049A3)P-2004-036:	\$118,000.	
FY 2009 Amendment 1 IA V101(049A3)P-2004-036:	\$ 34,335.	
FY 2009 Amendment 2 IA V101(049A3)P-2004-036:	\$ 9,000.	

Total Funding: \$ 161,335.

Deliverables:

- March 2007 Phase I Final Report and Appendices
- June 2007 Phase I presentation at the Department of Veterans Affairs
- October 2010 Interim Report on an evaluation of Antimicrobial Activity of Three Biocides on Marble
- November 2010 Interim Administrative Report
- December 2010 Report on an evaluation of Antimicrobial Activity of Three Biocides on Marble
- July 2011 Best Practices Recommendations Report
- July 2011 Final Results and Best Practice Recommendations Presentation to the National Cemetery Administration via Webex Teleconference
- October 2011 Chemical and Physical Analysis Report
- October 2011 Administrative Summary Report
- Publication: Jason Church, Mary Striegel, Christopher McNamara, Kristen Bearce Lee, and Ralph Mitchell, "Case Study: Comparative Study of Commercially Available Cleaners for Use on Marble Veterans Affairs Headstones" In Biocolonization of Stone: Control and Preventative Methods: Proceedings from the MCI Workshop Series, Edited by A. Elena Charola, Christopher McNamara, and Robert J. Koestler, Smithsonian Institution Scholarly Press, Washington, DC, 2011

Narrative:

In 2004 NCA and NCPTT entered into an interagency agreement to evaluate commercially available cleaners for use on federally-issued headstones. The goal of the research was to determine the effectiveness of the cleaners for removing biological growth and the length of time passing before regrowth was observed. Additional considerations included the ease of use of the treatment and the potential for long term stone damage. The work was carried out in the laboratory at NCPTT, through contractors at the Laboratory of Applied Microbiology, Harvard University, and in the field at five national cemeteries located in distinct geographic and climatic regions. Cemeteries included in this study were Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM.

Water and five commercially available cleaners, including Sunshine Makers Inc. D/2Antimicrobial cleaner, Certified Labs' Daybreak cleaner, World Environmental Group Marble cleaner, H2Orange Grout Safe cleaner, and Kodak Photo-Flo were evaluated at each test cemetery. Cleaners were applied to test patches on headstones carved from Colorado Yule marble and White Cherokee Georgia marble. Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations.

In field trials, changes to headstone test patches as a result of cleaning with test cleaners were evaluated by appearance change and biological activity. Laboratory studies looked for residual effects of cleaners including salt deposition that can lead to slow deterioration of the stone. Based on these results, two cleaners were eliminated from further consideration. Kodak Photo-Flo was a poor performer for the elimination of biological growth and did not inhibit re-growth on the headstones as evidenced in field studies. H₂Orange Grout Safe cleaner did not kill all microbes initially and left surface stains which vanished over time.

Field and laboratory studies continued on D/2 Antimicrobial cleaner, Daybreak cleaner, and World Monument Group Marble cleaner. Reoccurring biological activity was followed over eighteen month period in the field. Significant performance differences of these cleaners were not observed. Researchers associated with the project, including scientists at NCPTT and biologists at the Laboratory of Applied Microbiology, Harvard University, were concerned than an eighteen month time period may not have been sufficient to document significant visual changes or to allow for the growth of algae and photosynthetic bacteria. Laboratory tests indicated that D/2 Antimicrobial cleaner and Daybreak cleaner did leave soluble salts that could possibly affect long-term durability of the headstones.

Based on these results, the research project was extended through amendment 1 of the interagency agreement. The goal of additional research was to follow the reoccurrence of biological growth including bacteria, algae, and fungi on previously cleaned headstones in two cemeteries. Monitoring was to take place in Jefferson Barracks National Cemetery located in St. Louis, Mo. and in Santa Fe National Cemetery in Santa Fe, New Mexico. The two cemeteries were to be evaluated annually for a period of two years.

Due to a series of unforeseen events, headstones at Jefferson Barracks National Cemetery were cleaned without NCPTT's knowledge. Upon discovery by NCPTT staff at the first field evaluation, NCPTT and NCA consulted on alternatives to the field study.

In order to complete the project through amendment 2 of the interagency agreement, NCPTT developed an experimental design in collaboration with a contractor to determine in a series of microbiological analyses which of three biocides is most effective in the protection of marble against re-growth of microbial films.

They undertook research in collaboration with a contractor to analyze the effects of three biocides:

- 1. Sunshine Makers Inc D/2 Biological Solution,
- 2. Certified Laboratories Daybreak cleaner, and
- 3. World Environmental Group's Marble and Granite cleaner.

NCPTT staff prepared and provided marble samples and cleaners for the laboratory study. Analyses were carried out on Cherokee white marble and Colorado Yule marble. Each sample was analyzed for the presence of bacteria, fungi and algae at monthly intervals in a laboratory environment.

Based on the fundings of this research, NCPTT prepared recommendations on best practices to clean government issued veterans headstones .

Section 7

Interim Report

Institution/Organization: National Center for Preservation Technology and Training

Project Title: Comparative Study of Commercially-Available Cleaners for Federally-

Issued Headstones

Interagency Number: 101(049A3)P-2004-036

Summarize requested amendments (if any) to the original Grant Agreement or Work Cost/Budget and provide the approval date(s).

Funding History:

Original Agreement, August 24, 2004, \$118,000

Amendment 1, July 27, 2008, \$34,335

Amendment 2, April 15, 2009, \$9,000

Briefly describe progress to date for completing the project objectives as outlined in the Grant Agreement. Address each objective and associated task(s).

NCPTT's research was conducted in phases as described in the original interagency agreement.

1. Development phase

- a. Develop a research project that studies the effects of commercially available cleaning solutions on government-issued headstones. (Complete)
- b. Canvas cemetery stewards in private and VA national cemeteries to determine appropriate products/methods in current use for removal of biological growth on marble (Complete)
- c. Choose products for testing that are user friendly, suitable for large scale cleaning projects, environmentally friendly and cost effective. (Complete)

NCPTT worked closely with NCA staff to identify cleaners used in national cemeteries and choose commercially available products which were suitable for large scale cleaning projects. The number of cleaners was limited to five due to the number of biological test samples that could be performed over the time period of the project. Cleaners were chosen that covered a range of pH and chemical activity.

2. Phase I

a. Test approximately 1,440 headstones in five typical national cemeteries, within five NCA regions. Test eight products in side by side test patches on headstones. Testing is to take into consideration various orientations (i.e. east face, west face), various environments (i.e. full sun full shade), and other environmental conditions. (Complete)

b. Concurrent with test patch studies in the field, a series of cut marble samples will be treated with each of the eight products and exposed beside the test patch stones. These samples will be used in both non-destructive and destructive laboratory testing. The purpose of the testing will be to detect residual cleaning products on the stone and to look at potential stone deterioration. Analytical methods will be selected to detect chemical and physical changes to the surface of the cut test stones. (Complete)

The work was carried out in the laboratory at NCPTT, through contractors at the Laboratory of Applied Microbiology, Harvard University, and in the field at five national cemeteries located in distinct geographic and climatic regions. Cemeteries included in this study were Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM.

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3. Phase II (**redirected**)

- a. Based on the results of the test patches after at least nine months of study, up to four of the most effective cleaners will be further tested on whole headstones. Whole headstone studies will be monitored every three months for at least six months using the same techniques outlined for the patch study.
- b. Submit its findings and recommendations in a final report to NCA. The report will include experimental and analytical results with conclusions and recommendations as to future studies that would include a broader array of stone.

Phase II was eliminated in favor of longer term monitoring of the test patches. Due to the slow amount of biological re-growth in this study, NCPTT followed the field test patch studies for a much longer time frame than originally specified in the original interagency agreement.

Results of the study were presented in a detailed report, entitled "Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones" on March 10, 2007. The report and an oral presentation was given in June 2007. In this report, NCPTT recommended extending the study for an additional time frame of two years. Subtle appearance changes, the variability of biological growth, and the absence of algae supported this recommendation. Four options were offered to continue the project.

In July 2008, NCA and NCPTT signed amendment one to the interagency agreement to allow the research to continue for two more years.

The scope of NCPTT work defined in amendment one was as follows:

- 1. Continue research project by monitoring the re-growth of bacteria, algae, and fungi on headstones previously cleaned with one of five commercially available cleaners. Monitoring will take place in Jefferson Barrack National Cemetery located in St. Louis, Missouri and in Santa Fe National Cemetery, located in Santa Fe, New Mexico.
- 2. The two cemeteries will be evaluated annually for a period of two years. Evaluations will be based on color monitoring, changes in visual appearance, and biological analyses as provided by the laboratory of applied microbiology, Harvard University.
- 3. NCPTT will submit its findings and recommendations in a final report to NCA. The report will include experimental and analytical results with conclusions and recommendations as to future studies that would include a broader array of stone.

Originally, NCPTT planned to continue the study at Jefferson Barracks in St. Louis and at Pineville National Cemetery. Then, staff learned that Pineville NC had cleaned the headstones included in the study. NCPTT then proposed that the study be continued at Jefferson Barracks and Santa Fe National Cemetery. Santa Fe had very cooperative staff members who were interested in being part of the project. Also, Santa Fe National Cemetery was considered a best case scenario, since the environmental conditions were such that disfiguring biological growth was rare. This offered NCPTT scientists the chance to compare a low growth environment to a higher growth environment such as that found at Jefferson Barracks in St. Louis.

The first field campaign was scheduled and confirmed with Jefferson Barracks and Santa Fe National Cemetery in November 2008. Upon Jason Church's arrival at Jefferson Barracks, he learned that the headstones included in the study had been cleaned earlier in

the summer in preparation for visits by NCA officials. Jason continued to Santa Fe and collected data there.

On November 21, 2008, a conference call was held with NCA officials including Patrick Hallinan, Director of Field Programs, Lindee Lenox, Director, Memorial Programs Service, Sara Amy Leach, Senior Historian, Gina White, Program Analyst. NCPTT was represented by Mary F. Striegel and Jason W. Church. Since field data from Santa Fe National Cemetery alone would not provide significant information, a new strategy was recommended.

NCPTT proposed a laboratory study in partnership with Harvard University to look at the effectiveness of the three cleaners as biocides. The questions to be answered by this approach included: How do the remaining cleaners, including Daybreak, D2 Biological Solution, and World Environmental Group's Marble and Grout cleaner, perform as a biocide? Can these cleaners be differentiated based on how quickly biological growth returns on the stones?

NCPTT's Jason Church submitted a proposal and draft amendment two for the interagency agreement on January 28, 2009. Gina White Requested changes to the draft in March 2009. The agreement was executed on April 15, 2009. Unfortunately, the fully executed agreement was misplaced at the NCPTT office until June 30, 2009.

The scope of NCPTT work was redefined from amendment one, and in amendment two is as follows:

- 1. Develop an experimental design in collaboration with a contractor to determine in a series of microbiological analyses which of three biocides is most effective in the protection of marble against re-growth of microbial films. (Complete)
- 2. Undertake research in collaboration with a contractor to analyze the effects of three biocides:
 - a. Sunshine Makers Inc, D/2 Biological Solution
 - b. Certified Laboratories Daybreak Cleaner, and
 - c. World Environmental Group's Marble and Granite Cleaner. (Complete)
- 3. Prepare and provide marble samples and cleaners for the laboratory study. Analyses will be carried out on Cherokee White marble and Colorado Yule marble. Analyses will be carried out at each sampling time on five replicate samples of stone. Each sample will be analyzed for the preservation bacteria, fungi, and algae. Analyses will be undertaken at monthly intervals in a laboratory. (Complete)
- 4. Submit findings and recommendations in a final report to NCA. The report will detail the results of the laboratory study and summarize research efforts resulting from this interagency agreement. (In Progress)

What difficulties have you encountered to date in completing project work?

1. The work was complicated by the cleaning of headstones in the selected cemeteries. This lead to a change of approach in the research.

- 2. Delays from April to July 2009 were encountered due to the misplacement of the second amendment in our offices.
- 3. Unexpected delays from federal procurement procedures slowed the award of the contract to Harvard University.
- 4. The microorganisms did not respond as expected in the laboratory studies. A significant amount of time was needed to develop better culture methods to get appropriate microorganisms to grow in the lab. Once the culturing methods were perfected, scientists had to wait for the microorganisms to grow. Then samples were cleaned with the specified cleaners. Researchers are now studying the re-growth patterns, which has been slow. This has resulted in unavoidable delays in the final results.

What changes in objectives or budget or products are anticipated?

No further changes in the objectives of the study are expected. The main contractor on the project is Dr. Ralph Mitchell, who has expressed need for further funding should the project continue beyond the end of the calendar year. A final report and recommendations will be completed within 30 days of the completion of the experimental work.

Will you be able to complete work under this interagency agreement as scheduled? If not, why?

Tests are continuing to distinguish between three biocides on marble samples. To date, 127 days have passed since the samples have been cleaned. Results of the study are detailed in the interim report (attached). We are continuing to follow regrowth which is dependent on the microorganisms.

What products (if any) have been produced to date?

Reports

Analysis of Microorganisms on Headstones in VA Cemeteries, December 2005, by Ralph Mitchell, Kristen Bearce and Christopher McNamara, Laboratory of Applied Microbiology, Division of Engineering and Applied Sciences, Harvard University.

Analysis of Microorganisms on Headstones in VA Cemeteries, Second Report, June 2006, by Ralph Mitchell, Kristen Bearce and Christopher McNamara, Laboratory of Applied Microbiology, Division of Engineering and Applied Sciences, Harvard University.

Analysis of Microorganisms on Headstones in VA Cemeteries, Third Report, February 2007, by Ralph Mitchell, Kristen Bearce and Christopher McNamara, Laboratory of Applied Microbiology, Division of Engineering and Applied Sciences, Harvard University.

Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones, Progress Report, Progress Update as of March 10, 2007, by Mary F. Striegel, and Jason W. Church, NCPTT.

Interim report on an evaluation of antimicrobial activity of three biocides on marble, October 2010 by Ralph Mitchell, Archana Vasanthakumar, Conor O'Herin, and Alice DeAraujo, (**Attached**)

Presentations

Comparative Study of Commercially Available Cleaners for Use on Federally Issued Headstones, by Georgette Lang, Student presentation, Preservation In Your Community, Natchitoches, La., August 3, 2006.

Comparative Study of Commercially Available Cleaners for Use on Federally Issued Headstones, by Mary F. Striegel to the National Cemetery Administration, Washington, DC, June 7, 2007.

Comparative Study of Commercially Available Cleaners for Use on Federally Issued Headstones, by Lauren Vienne, Student presentation, Preservation In Your Community, Natchitoches, La. August 8, 2007.

Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones, By Jason W. Church, Association for Preservation Technology International, Montreal, Canada, October 13-17, 2008.

Comparative Study of Commercially Available Cleaners for use on Federally-Issued Headstones, By Jason W. Church, Biocolonization of Stone Workshop, Smithsonian's MCI Center, Camp Springs, MD, April 21, 2009.

Comparative Study of Commercially Available Cleaners for use on Federally-issued Headstones, By Jason W. Church, Nationwide Cemetery Preservation Summit, Nashville, Tenn., October 19-21, 2009.

Comparative Study of Commercially Available Cleaners for use on Federally-Issued Headstones, By Jason W. Church, poster presentation at DOI Conference on the Environment, Portland, OR, April 28, 2010.

What products (if any) are currently underway?

Experiments on the antimicrobial activity of three biocides on marble are continuing, but will be expected to finish by December 31, 2010. Upon completion of the experiments, a final report will be prepared and submitted.

The completion date for the final report is January 30, 2011.

Signature principal investigator: Mary 7 Strigger

Date: <u>November 19, 2010</u>

An interim report on an evaluation of antimicrobial activity of three biocides on marble

To

Jason Church

NCPTT, National Park Service

Investigators:

Ralph Mitchell, Gordon McKay Professor of Applied Biology Archana Vasanthakumar, Postdoctoral Fellow Conor O'Herin, Undergraduate researcher Alice DeAraujo, Research assistant

School of Engineering and Applied Sciences Harvard University

October 2010



Objectives

In a laboratory study, we used disks of Colorado and Georgia marble to test the ability of three biocides, DaybreakTM, D/2 and WEG marble cleaner, to resist the challenge by a high concentration of microorganisms. This interim report describes the results after 127 days of testing in a high humidity environment at a temperature of 72°F.

Methods

Experimental set up

Two types of marble, Colorado and Georgia, were used in this study. Marble disks were heatsterilized before the experiment was set up. A fungus, *Aspergillus niger*, was chosen for inoculation on the marble surface, as our indicator of biocidal activity.

Fifty microliters of a microbiological growth medium were placed on the surface of each disk. Once the media air dried, 20 µl of fungal suspension was added to each disk. Disks inoculated with fungi were placed in plant growth trays (Figure 1), which in turn were placed on a flat tray containing water. Clear plastic domes were used to cover the plant growth trays, thereby helping to keep the humidity high. All inoculated disks were maintained at high humidity and at room temperature (ca. 72°F) for a total of 7 weeks before treatment with biocides.

The day before biocide treatment, three disks for each marble type were set aside as controls.

These were marked positive controls and were not treated with biocides.



Figure 1: Experimental set-up showing marble disks arrayed in a plant growth tray.

Sampling microbial populations

Disks were swabbed in order to obtain a baseline estimate of the concentration of microorganisms before cleaning. A high concentration of microorganisms was detected before biocidal treatment. Sterile swabs were used to sample the surface of inoculated marble disks. Swab samples were then dipped into 0.5 ml sterile water so that microorganisms could be transferred to the water. Fungi were quantified using a rapid fluorimetric assay developed in our laboratory.

Biocides

Three biocides, D/2, Daybreak[™] and World Environment Group's (WEG) marble cleaner, were tested. D/2 is an architectural biocide. Daybreak[™] is a mildew stain remover. The WEG marble cleaner is a soy-based, environmentally friendly cleaner. Appropriate concentrations of the three biocides were made up in the sprayers provided. Inoculated disks were treated in the laminar flow hood with a quick 2-second spray onto the top surface (the inoculated surface) of each disk. Two rounds of spraying were performed with 1 min between sprays. About 3 minutes after the 2nd round of spraying, paper towels were used to mop up excess liquid on the surface of disks.

This process was repeated for each cleaner. Once the disks were "dry", they were transferred back to the plant growth trays in order to maintain high humidity. They were then incubated at room temperature. The relative humidity was ca. 80%. Representative disks were sampled on day 1 (the day after biocide treatment) as well as at the sampling times shown in figures 2 and 3.

Results

We tested the effectiveness of three biocides, D/2, Daybreak[™] and WEG marble cleaner in preventing growth of microorganisms on Colorado and Georgia marble. We sampled the disks for microbial growth at the following times, after spraying with the biocides:, Day 1, Day 34, Day 41, Day 90 and Day 127. The effects of the biocides in the control of microbial growth on Colorado marble are shown in Figure 2. The results for Georgia marble are shown in Figure 3.

Soon after biocide treatment (Day 1 sampling time), almost no fungi were detected on the treated stone disks. The biocides had reduced the microbial population to nearly zero. In contrast, the microbes on the untreated disks continued to grow well. All three biocides controlled the biological activity equally. At days 34 and 41, higher fungal populations, relative to Day 1, were detected. The fungal population slowly increased with time, as shown in Figures 2 and 3, though it was still very low compared to initial populations. After 127 days, there was no apparent difference among the activity of the three biocides. The study is continuing with projected sampling times extending to 6 months after biocide treatment.

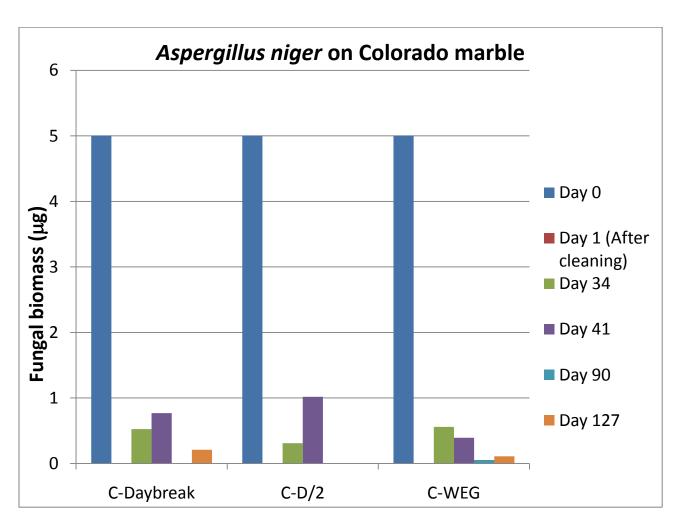


Figure 2: Effects of the three biocides, DaybreakTM, D/2 and WEG marble cleaner on fungal growth on Colorado marble

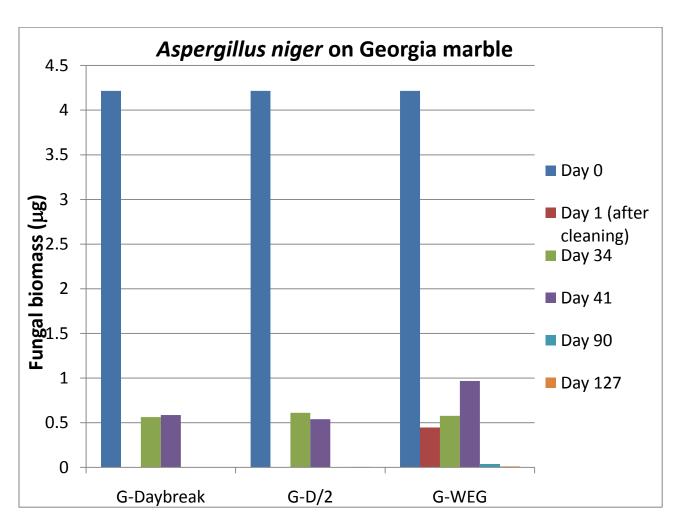


Figure 3: Effects of the three biocides, DaybreakTM, D/2 and WEG marble cleaner on fungal growth on Georgia marble

Interim conclusions

We tested the ability of three biocides, D/2, DaybreakTM and WEG marble cleaner, to control microbial growth on Colorado and Georgia marble under accelerated laboratory conditions. The temperature was maintained at 72F and the humidity at 80%. Marble disks were inoculated with the microorganisms in a growth medium and treated with the biocides. We used mold (fungi) growth as an indicator. All three biocides prevented growth equally for 127 days. The tests are continuing in order to determine which of the three biocides is most protective.

Section 8



YOUR AMERICA

Comparative Study of Commercially Available Cleaners for Use on Federally Issued Headstones

Mary F. Striegel
Jason W. Church

Funded by the Department of Veterans Affairs, National Cemetery Administration June 7, 2007



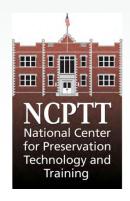


YOUR AMERICA

Introduction



- The National Cemetery Administration maintains 3.6 million gravesites.
- Loss of legibility or deteriorating conditions lead to headstone replacement.



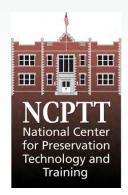


YOUR AMERICA

Maintenance Issues



- Cleaning may accelerate stone weathering
- Unsuitable cleaners can damage stone by:
 - Loss of surface
 - Staining
 - Deposition of soluble salts
 - Make the stone more vulnerable to pollution or biological growth

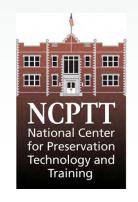




YOUR AMERICA

The Salt Issue

- Soluble salts collect in pores of the stone
- As the environment cycles, the salts alternate between a liquid and solid phase
- Salt crystals grow in size, rupturing the pores.





YOUR AMERICA

The Ideal Cleaner

- ✓ Does no harm.
- ✓ Gentlest and least invasive method.
- ✓ Leaves acceptable appearance.
- ✓ Does not leave salts behind.
- ✓ Does not alter the chemistry of the stone surface.
- ✓ Should be a similar pH to the stone.
- ✓ Does not alter physical properties of the stone, such as surface roughness or porosity.
- ✓ Slows biological re-growth.
- ✓ Easy to use.



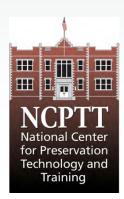


YOUR AMERICA

Goals of Project



- Evaluate commercially available cleaners based on:
 - Appearance
 - Biological Re-growth
 - Physical Changes
 - Chemical Changes
 - Ease of Use

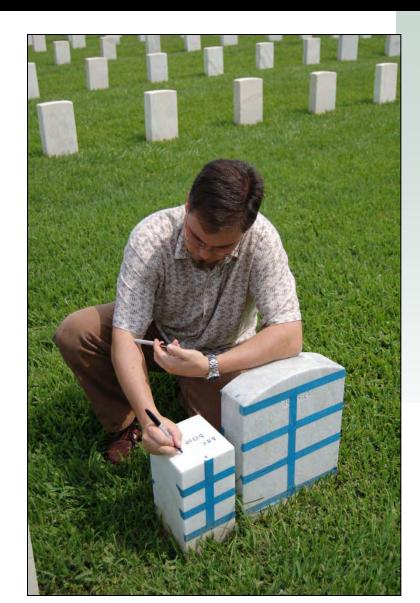




YOUR AMERICA

Methodology

- Field Studies including different:
 - Climates
 - Environments
 - Stone types
- Measuring:
 - Color
 - Biological activity
 - Visual appearance





YOUR AMERICA

Methodology

- Laboratory Studies including:
 - Artificial Weathering
 - Macro- and Microanalytical techniques
- On:
 - Laboratory marble samples
- Measuring:
 - Chemical Changes
 - Physical Changes





YOUR AMERICA

Selection of Cemeteries

- Different Geographic Regions
- Different Climatic Zones
 - Koppen Climate classification System





San Francisco National Cemetery

• San Francisco, CA

Bath National Cemetery

• Bath, NY

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Jefferson Barracks National Cemetery

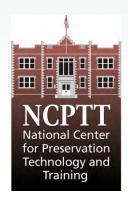
• St. Louis, MO

Santa Fe National Cemetery

• Santa Fe, NM

Alexandria National Cemetery

• Pineville, LA





Alexandria National Cemetery

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- Pineville, LA
- First Burial:1867
- Zone Cfa, Humid Subtropical
 - Mild climate
 - No dry season
 - Hot summers

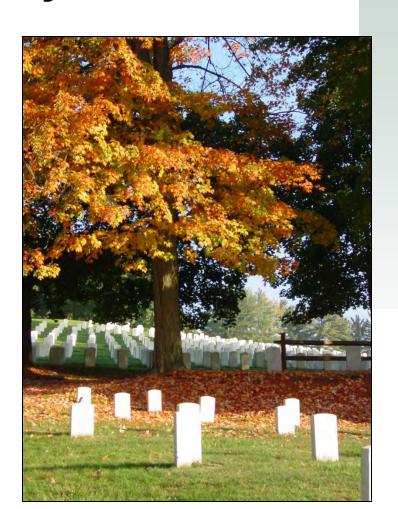




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Bath National Cemetery

- Bath, NY
- First Burial: 1879
- Zone Dfb, Humid Continental
 - humid climate with severe winter
 - No dry season
 - Warm summers





Jefferson Barracks National Cemetery

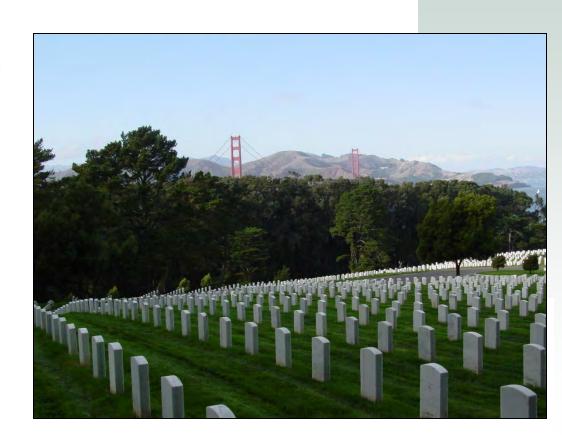
- St. Louis, MO
- First Burial: 1827
- Zone Dfa, Humid Continental
 - Humid, cold climate
 - No dry season
 - Hot summers





San Francisco National Cemetery

- San Francisco, CA
- First Burial: 1850
- Zone Csb, Mediterranean
 - Temperate, wet winters
 - Warm or hot summers





Santa Fe National Cemetery

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- Santa Fe, NM
- First Burial: 1868
- Zone Bsk,
 Semi-Arid Steppe
 - Hot summers and Cold winters
 - Similar to a praire





Selection of Cleaners: Cleaners Considered, see handout, table 1.

Product Name							
	acidic	basic	alcohol	chelate	solvent	surfactant	bactericide
Stone Quest							
Stone Care International				X		X	
GK125							
Geokleen Inc.					X		
Multi Surface Cleaner							
1000							
World Environmental							
Group, Inc.			X	X		X	
Omni-Green							
National Plastics and							
Chemical Corp.							
Stone-Kleen							
Mid Atlantic Chemical	X						X
H ₂ Orange ₂ Grout Safe							
Proven Solutions	X						
Hurricane Intensive Stone							
Cleaner							
National Chemical							
Laboratories			X		X		
Zep-A-One							
Zep Manufacturing, Co.				X	Χ	X	
Marble Cleaner							
World Environmental							
Group, Inc		X	X	Х		X	
Kandu #110							
SpaceAge Coating							
Concepts, Inc.		X					
Daybreak							
NCH Corporation,							
Certified Labs		X					
D/2							
Sunshine Makers, Inc.						X	X
Sodium Bicarbonate							
Kodak Photo-Flo							
Kodak Corporation			X		X	X	
Hypo Clear							
Kodak Corporation				X		X	X





Selection of Cleaners: Cleaners Chosen, see Handout, Tables 2 & 3.

	pН	Acidic	Basic	Alcohol	Chelate	Solvent	Surfactant	Bactericide
D-2	9.5						X	X
Daybreak H2Orange2 Grout	12.1		X					
Safe	3.81	X						
Kodak Photo-Flo	7			X		X	X	
Marble Cleaner	10.5		X	X	X		X	

Cleaner	H2Orange2 Grout Safe	Kodak Photo-Flo	D-2	Marble Cleaner	Daybreak
рН	3.81	7	9.5	10.5	12.1

Table 1. Chosen Cleaners are ordered from Acidic to Basic.



YOUR AMERICA

Field Trials

- Photographic documentation
- Initial biological activity
- Cleaning test patches
- Appearance changes
- Follow-up biological re-growth







YOUR AMERICA

Lab Trials

- Artificial Weathering
- Field Stones for Lab Testing
 - Laser Profilometry
 - Stone Porosity
 - Optical Microscopy
 - X-ray FluorescenceAnalysis
 - Total Soluble Solids





Appearance







Biological Re-growth







Jefferson Barracks



Biological Re-growth







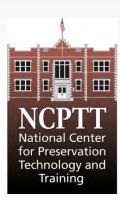
San Francisco



Biological Re-growth

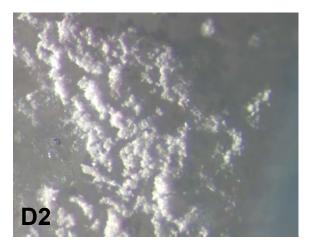


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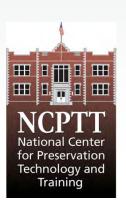
Physical Changes







 Salt growth documented on lab samples using optical microscopy EXPERIENCE YOUR AMERICA



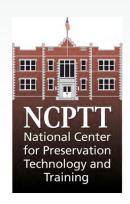


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Chemical Changes (In-Progress)



- Evaluating Possible Cleaner Residues
 - on field test stones
 - Lab stones from artificial weathering
- Using
 - X-ray Fluorescence
 - Electron Microscopy
 - Powder X-rayDiffraction





Related Maintenance Issues EXPERIENCE YOUR AMERICA





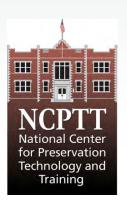


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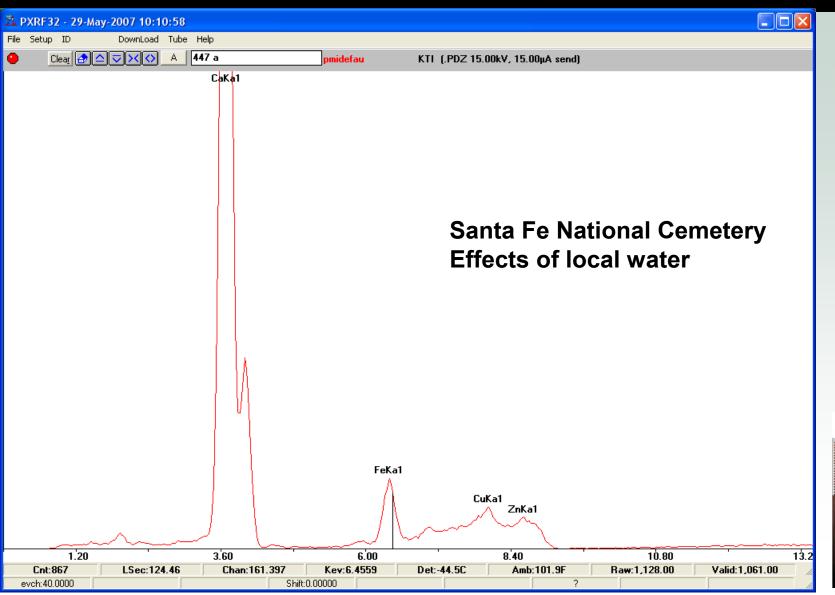
Chemical Changes



- Concerns about cleaning in Santa Fe National Cemetery
 - Yellowing of stone seen over time
- Regular Irrigation
 - Iron deposits from water







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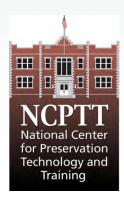


Issues Associated With Bath National Cemetery











Recommendations regarding Bath NY

- Eliminate Bath National Cemetery from Study
- Contamination of stone surfaces
 - Dirt
 - Hydro-seeding
- Loss of study headstones
 - Breakage during resetting



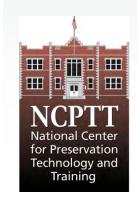




YOUR AMERICA

Results to date

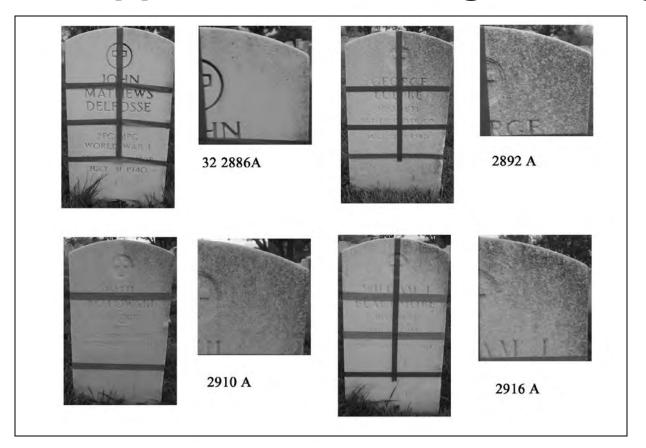
- Appearance changes
 - Visual Changes: Biological Re-growth
 - Color Measurement
 - Sunny vs Shady Areas
- Biological Activity
 - Biological Counts
 - Biological Activity by Location
 - Biocidal Performance
- Artificial Weathering

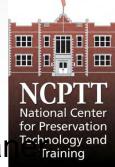




EXPERIENCE

Visual Appearance: Biological Re-growth POUR Appearance





Jefferson Barracks Test Patches: Note H2Orange2 Clear

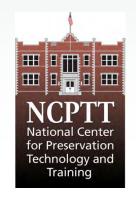


YOUR AMERICA

Color Measurement

Cleaner	Freq dE> 5	Freq dE> 10
D/2	5.00	1.00
Daybreak	7.00	2.00
Water	7.00	2.00
H2Orange Cleaner	5.00	2.00
WEG Marble Cleaner	8.00	1.00
Kodak Photo-flo	11.00	3.00

 Kodak Photo-Flo shows the greatest number of color changes for both categories.





Color Measurement: Sunny vs. Shady

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Shady			Sunny		
Cleaner	Freq dE> 5	Freq dE> 10	Cleaner	Freq dE> 5	Freq dE> 10
D/2	2.00	0.00	D/2	3.00	1.00
Daybreak	4.00	2.00	Daybreak	3.00	0.00
Water	3.00	2.00	Water	4.00	0.00
H2Orange Cleaner	3.00	1.00	H2Orange Cleaner	3.00	1.00
WEG Marble Cleaner	4.00	1.00	WEG Marble Clean	4.00	0.00
Kodak Photo-flo	6.00	2.00	Kodak Photo-flo	5.00	1.00
	22.00	8.00		22.00	3.00

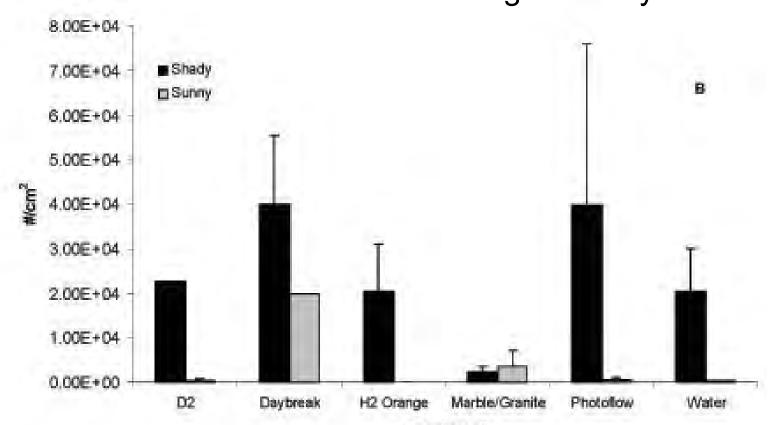
- This data indicates that there is an equal chance of seeing color change in sunny and shady areas for color changes greater than ∆E>5
- Greater chance of seeing major color change in shady location.





Biological Activity

Jefferson Barracks Fungal Activity



Treatment

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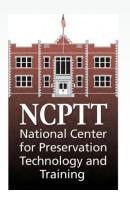




YOUR AMERICA

Biological Activity by Location

- 1. Bath
- 2. Jefferson Barracks
- 3. San Francisco
- 4. Alexandria
- 5. Santa Fe





Biocidal Performance

YOUR AMERICA

VA Samp	oles					Harva	rd Bioanaly	sis				Ju	ine 2006
Appendix, Biolog	ical Perfor	nance Bas	ed on Sun	or Shade									
	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	BShady	FShady	Shade Overall	Bshade	Fshade
D2	4	1	4	1	5	3	4	1	4	6	3.3	4.2	2,4
Daybreak	5	6	4	6	4	1	1	5	1	5	3.8	3	4.6
H2Orange	1	6	5	3	4	3	3	6	3	5	3.9	3.2	4.6
Marble/Granite	6	6	3	4	4	6	5	3	2	1	4	4	4
Photo-flo	3	5	1	4	3	1	2	6	3	6	3.4	2.4	4.4
Water	2	5	3	5	1	3	4	4	3 2	6	3.5	2.4	4.6
	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	BSunny	FSunny	Sun Overall	Bsun	Fsun
D2	3	4	4	1	4	6	3	6	5	6	4.2	3.8	4.6
Daybreak	3	6	1	4	3	1	5	3	6	4	3.6	3.6	3.6
H2Orange	3	5	6	5	1	6	1	6	6	1	4	3.4	4.6
Marble/Granite	4	1	4	3	5	5	2	4	6	5	3.9	4.2	3.6
Photo-flo	1	2	6	3	1	6	2	1	1	5	2.8	2.2	3.4
Water	6	6	2	6	2	6	3	5	6	6	4.8	3.8	5.8
	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotGrowth	TotOverall	Boverall	Foverall
D2	3.5	2.5	4	1	4.5	4.5	3.5	3.5	4.5	6	3.75	4	3.5
Daybreak	4	6	2.5	5	3.5	1	3	4	3.5	4.5	3.7	3.3	4.1
H2Orange	2	5.5	5.5	4	2.5	4.5	2	6	4.5	3	3.95	3.3	4.6
Marble/Granite	5	3.5	3.5	3.5	4.5	5.5	3.5	3.5	4	3	3.95	4.1	3.8
Photo-flo	2	3.5	3.5	3.5	2	3.5	2	3.5	2	5.5	3.1	2.3	3.9
Water	4	5.5	2.5	5.5	1.5	4.5	3.5	4.5	4	6	4.15	3.1	5.2



YOUR AMERICA

Artificial Weathering

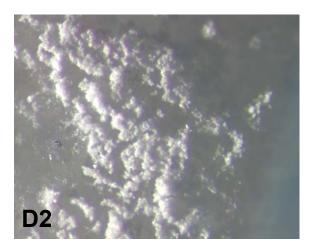
- Tested Colorado Yule Marble
- D2, Daybreak, WEG Marble Cleaner, Water
- 800 hours of exposure to UV light
- Cycles of
 - 4 hours light
 - 4 hours dark w condensation





YOUR AMERICA

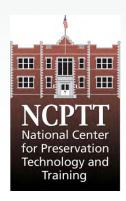
Physical Changes







 Salt growth documented on lab samples using optical microscopy





YOUR AMERICA

Elimination of Cleaners

- Eliminated Kodak Photo-flo based on
 - Biocidal performance rankings
 - Color changes seen in sunny and shady areas
- Eliminated H2Orange2 Grout Cleaner based on:
 - Biological re-growth, ex. Jefferson Barracks





YOUR AMERICA

Recommendations to Date

- Wet the stone prior to cleaning
 - Keeps the cleaner on the surface of the stone
- Apply chemical cleaner
 - WEG Marble Cleaner, D2, or Daybreak,
- Agitate if necessary (Spot cleaning)
- Rinse thoroughly with lots of water
 - To prevent salt deposition and growth





YOUR AMERICA

In-progress

- Laboratory testing on field test stones for physical and chemical changes
- Analysis of whole headstone cleaning
- Field evaluation of surface changes
- Identification of salt formation on artificially weathered stone





YOUR AMERICA

Reasons for Continuation of Study

- Changes to headstones evaluated based on 12-18 months of field exposure
- All headstones display a relatively small biofilm
- Variability was observed in biological regrowth
- No algae were present: too early for regrowth
- Visual appearance changes were subtle





YOUR AMERICA

Option A (\$52,800)

- Evaluate four cemeteries
 - Alexandria, Jefferson Barracks, San Francisco and Santa Fe
- One trip a year to evaluate test patches and whole headstones for two years
 - Analyze biological activity
 - Observe visual changes
 - Measure color changes
 - Document surfaces using digital microscopy





YOUR AMERICA

Option B (\$31,600)

- Evaluate two cemeteries
 - Alexandria, and Jefferson Barracks,
- One trip a year to evaluate test patches and whole headstones for two years
 - Analyze biological activity
 - Observe visual changes
 - Measure color changes
 - Document surfaces using digital microscopy





YOUR AMERICA

Option C (\$14,100)

- Evaluate one cemetery
 - Alexandria
- Two trips a year to evaluate test patches and whole headstones for two years
 - Analyze biological activity
 - Observe visual changes
 - Measure color changes
 - Document surfaces using digital microscopy

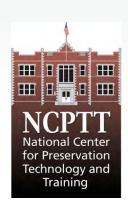




YOUR AMERICA

Option D (\$6,100)

- Evaluate one cemetery
 - Alexandria
- Two trips a year to evaluate test patches and whole headstones for two years
 - No biological activity
 - Observe visual changes
 - Measure color changes
 - Document surfaces using digital microscopy





YOUR AMERICA

Acknowledgements

- National Cemetery Administration
 - Dave Schettler
 - Sara Amy Leach
 - Karen Ashton
- Laboratory of Microbial Ecology, Harvard University
 - Dr. Ralph Mitchell
 - Kristen Bearce





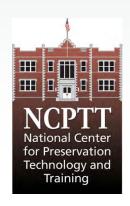
YOUR AMERICA

Acknowledgements

- Alexandria National Cemetery
 - J.D. Murray, Supervisor Natchez Cemetery

- Bath National Cemetery
 - Skip Baroody, Superintendent

- Jefferson Barracks National Cemetery
 - Ralph Church, Superintendent
 - Terry Smart, Grounds Supervisor





YOUR AMERICA

Acknowledgements

- San Francisco National Cemetery
 - Genaro Ocrato, Golden Gate Cemetery
 - Ron Wonalowski, Superintendent Golden Gate Cemetery
 - Mike Tyne, Contractor Dial General Engineering

Santa Fe National Cemetery

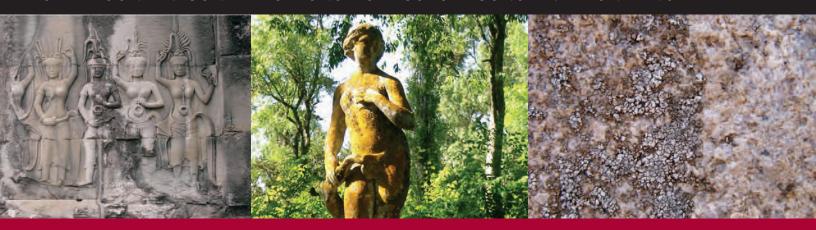
- Wesley Jones, Superintendent
- Joseph Lovato, Grounds Supervisor



Section 9



SMITHSONIAN CONTRIBUTIONS TO MUSEUM CONSERVATION • NUMBER 2



Biocolonization of Stone: Control and Preventive Methods

Proceedings from the MCI Workshop Series

Edited by
A. Elena Charola,
Christopher McNamara,
and Robert J. Koestler

SERIES PUBLICATIONS OF THE SMITHSONIAN INSTITUTION

Emphasis upon publication as a means of "diffusing knowledge" was expressed by the first Secretary of the Smithsonian. In his formal plan for the Institution, Joseph Henry outlined a program that included the following statement: "It is proposed to publish a series of reports, giving an account of the new discoveries in science, and of the changes made from year to year in all branches of knowledge." This theme of basic research has been adhered to through the years by thousands of titles issued in series publications under the Smithsonian imprint, commencing with Smithsonian Contributions to Knowledge in 1848 and continuing with the following active series:

Smithsonian Contributions to Anthropology Smithsonian Contributions to Botany Smithsonian Contributions to History and Technology Smithsonian Contributions to the Marine Sciences Smithsonian Contributions to Museum Conservation Smithsonian Contributions to Paleobiology Smithsonian Contributions to Zoology

In these series, the Institution publishes small papers and full-scale monographs that report on the research and collections of its various museums and bureaus. The Smithsonian Contributions Series are distributed via mailing lists to libraries, universities, and similar institutions throughout the world.

Manuscripts submitted for series publication are received by the Smithsonian Institution Scholarly Press from authors with direct affiliation with the various Smithsonian museums or bureaus and are subject to peer review and review for compliance with manuscript preparation guidelines. General requirements for manuscript preparation are on the inside back cover of printed volumes. For detailed submissions requirements and to review the "Manuscript Preparation and Style Guide for Authors," visit the Submissions page at www.scholarlypress.si.edu.

Biocolonization of Stone: Control and Preventive Methods

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Edited by
A. Elena Charola,
Christopher McNamara,
and Robert J. Koestler



ABSTRACT

Charola, A. Elena, Christopher McNamara, and Robert J. Koestler, editors. *Biocolonization of Stone: Control and Preventative Methods, Proceedings from the MCI Workshop Series.* Smithsonian Contributions to Museum Conservation, number 2, 116 pages, 87 figures, 5 tables, 2011. — The Smithsonian Museum Conservation Institute Workshop on Biocolonization of Stone was the second workshop in a series and was dedicated to research on removal and control of biocolonization in stone objects. Twelve presentations were made, and the workshop ended with a roundtable discussion open to the 71 attendees. The goal was to provide a discussion forum for biologists, material scientists, and conservators interested in stone biodeterioration. Seven papers were presented, ranging from microbiological laboratory studies to combination of on-site testing and laboratory evaluation for World Heritage Sites such as Angkor Wat, to a literature overview. Five case studies were also presented, covering control of biodeterioration at Veterans Affairs cemeteries, experience gathered from the installation of zinc strips at the Stanford Mausoleum in San Francisco, the red staining found on the marble of the Memorial Amphitheater at Arlington National Cemetery, problems posed by deer stones in Mongolia, and the site test installed at San Ignacio Miní Jesuit mission in Misiones, Argentina. The roundtable and discussions drew attention to the importance of exploring new methods to prevent microbial colonization of stone. Finally, in a closed session, suggestions were offered for developing criteria to evaluate microbial growth and determine when treatment is necessary. It was recommended that a database be prepared on stone biocolonization and its control.

Cover images, from left to right: Details of Figure 5b by Warscheid and Leisen and Figure 1b by Delgado et al.; Figure 8 by DePriest and Beaubein.

Published by SMITHSONIAN INSTITUTION SCHOLARLY PRESS

P.O. Box 37012 MRC 957 Washington, D.C. 20013-7012 www.scholarlypress.si.edu

Library of Congress Cataloging-in-Publication Data

Smithsonian Museum Conservation Institute Workshop on Biocolonization of Stone: Control and Preventive Methods (Washington, D.C.: 2009)

Biocolonization of stone: control and preventive methods: proceedings from the MCI workshop series / edited by A. Elena Charola, Christopher McNamara, and Robert J. Koestler.

p. cm. — (Smithsonian contributions to museum conservation, ISSN 1949-2359; no. 2) Includes bibliographical references.

1. Museum conservation methods—Congresses. 2. Stone—Biodegradation—Congresses. 3. Microbiologically influenced corrosion—Congresses. 4. Cleaning—Environmental aspects—Congresses. 5. Museums—Collection management—Congresses. I. Charola, A. Elena. II. McNamara, Christopher J. III. Koestler, Robert J. (Robert John), 1950–IV. Museum Conservation Institute. V. Title.

AM145.S648 2009 069'.53—dc22

2010052916

ISSN (print): 1949-2359 ISSN (online): 1949-2367

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Case Study: Comparative Study of Commercially Available Cleaners for Use on Marble Veterans Affairs Headstones

Jason Church, Mary Striegel, Christopher McNamara, Kristen Bearce Lee, and Ralph Mitchell

variety of commercial products are available for use in cleaning stone surfaces contaminated with microbiological growth. The effectiveness of many of these products is questionable, however, and direct comparison of some commonly used products would be of significant interest to conservators. Therefore, a study was carried out to compare commercially available cleaners for the removal of soiling and biological growth from federally issued headstones. Specific goals were to test cleaning products for effectiveness to recommend those products and methods best suited to clean and preserve headstones.

The study focused on five national cemeteries: Alexandria National Cemetery in Pineville, Louisiana; Bath National Cemetery in Bath, New York; Jefferson Barracks National Cemetery in St. Louis, Missouri; San Francisco National Cemetery in San Francisco, California; and Santa Fe National Cemetery in Santa Fe, New Mexico. These cemeteries were chosen to represent the various regions of the National Cemetery Administration (NCA) as well as different climatic zones, including subtropical, temperate, continental, semiarid, and oceanic climates. Stones that were tested in the cemeteries were carved from the Colorado Yule marble and Georgia White Cherokee marble used for the majority of both modern and historic federally issued headstones.

Prior to cleaning, baseline biological activity was documented on test areas in the fall of 2005. Headstones were then evaluated 6 and 12 months after cleaning. Tap water from the site and five commercially available cleaners were selected for application to test areas on 48 headstones at each cemetery. Products were chosen to include cleaners that are frequently used, environmentally friendly, user friendly, and unlikely to damage the stone. Daybreak (NCH Corp., Certified Labs), based on sodium hypochlorite, is the most commonly used cleaner within the NCA. Kodak Photo-Flo (Kodak Corp.), a mixture of p-tert-octylphenoxy polyethoxyethyl alcohol and propylene glycol, has been

Jason Church and Mary Striegel, National Center for Preservation Technology and Training, 645 University Parkway, Natchitoches, Louisiana 71457, USA. Christopher McNamara, Harvard School of Public Health, Harvard University, Boston, Massachusetts 02215, USA. Kristen Bearce Lee and Ralph Mitchell, Laboratory of Applied Microbiology, Harvard School of Engineering and Applied Sciences, Harvard University, 40 Oxford Street, Cambridge, Massachusetts 02138, USA. Correspondence: M. Striegel, mary_striegel@nps.gov.

commonly used to clean headstones following the recommendation by Strangstad (1988), presumably because it promotes faster drying. The three remaining cleaners were chosen to provide a range of compositions. H₂Orange, Grout Safe (Proven Solutions Inc.) contains hydrogen peroxide in a slightly acidic solution. D/2 Architectural Biocide (Sunshine Makers Inc.), mainly containing quaternary ammonium compounds, has antimicrobial properties. Marble Cleaner (World Environmental Group) has cleaning properties based on the action of surfactants and chelating agents. Cleaners were spray applied to test patches measuring approximately 6 × 6 inches (approximately 15 × 15 cm) on 20 headstones at each site. Each solution was applied to stones in both sun-exposed and shaded locations to account for possible differences arising from local environmental variations.

Headstone test patches were evaluated for changes in appearance after 6 months and biological activity after 6 and 12 months. Appearance changes were documented using photography and color measurements. The color data were collected with a Minolta CR-400 Colorimeter (Figure 1), measuring three spots on each test area and averaged for a total of 18 measurements per headstone. The

same areas were measured in each case and evaluated by calculating the number of color changes where the total change (DE) was greater than 5 points, which is perceptible to the human eye.

A 3 × 3 cm area of the headstone surface was sampled for microorganisms using BBL Liquid Amies Culture Swabs (Becton-Dickinson). To quantify microbial growth, heterotrophic bacteria and fungi were enumerated by plating samples on solid media (nutrient agar and malt extract agar, respectively). Plates were incubated at room temperature for two days, and colonies were counted. Cyanobacteria and algae were enumerated using a hemocytometer. Performance of test cleaners was compared on the basis of biological regrowth activity by ranking the cleaners from 1 to 6. The ranking of 1 was given to the cleaner that had the highest regrowth rate, and 6 was given to the cleaner with the lowest regrowth rate. Thus, lower numbers indicate more poorly performing cleaners.

Algae and cyanobacteria were not observed on any of the headstones prior to cleaning, but other bacteria and fungi were detected in almost all locations by sampling using the Liquid Amies Culture Swabs. Numbers of organisms varied greatly among cemeteries and headstones, and



FIGURE 1. Jason Church takes color measurements of a headstone after cleaning.

numbers of bacteria on the headstones (~10⁵/cm²) generally averaged one or two orders of magnitude greater than the numbers of fungi (~10³/cm²).

After six months, the cleaned areas showed the greatest number of changes in color measurements ($\Delta E > 5$ and 10) on test patches cleaned with Kodak Photo-Flo (Table 1). No algae or cyanobacteria were observed in samples collected after six months, but numbers of other bacteria and fungi were generally consistent with the color measurements for specific cleaners. The lowest levels of growth, for example, were often observed in samples that had been treated with D/2 and Daybreak, which performed best according to the color measurements. The numbers of bacteria (10⁷–10⁸/cm²) and fungi (10³–10⁴/cm²) were generally higher than in the initial samples, however, most likely because of seasonal effects. Samples collected in November and December could be expected to have less biological material than samples collected in the spring months of April and May.

On the basis of the data obtained for appearance change and biological activity, Kodak Photo-Flo was eliminated from further testing after six months. H₂Orange₂ Grout Safe cleaner performed well according to color measurements of biological activity after six months, but closer inspection of headstones indicated that biological staining was present on some test areas. On the edges of headstones at Jefferson Barracks, for example, activity is clearly visible near the edges away from areas measured for color change (Figure 2). On the basis of these observations of growth, H₂Orange₂ Grout Safe was also eliminated from the study.

Biological activity evaluations after 12 months did not detect algae and cyanobacteria on areas treated with the four remaining solutions (D/2, Daybreak, Marble Cleaner, and water). These organisms typically provide the most visual evidence of growth on headstones, and their absence,

TABLE 1. Number of color change measurements (ΔE) greater than 5 and 10 for each cleaner applied on headstones at Alexandria, Jefferson Barracks, San Francisco, and Santa Fe National Cemeteries.

Cleaner	$\Delta E > 5$	$\Delta E > 10$
D/2	5	1
Daybreak	7	2
H ₂ Orange ₂	5	2
H ₂ Orange ₂ Marble Cleaner	8	1
Photo-Flo	11	3
Water	7	2

even from stones treated with water, suggests that a 12 month period may be too short for determination of the effectiveness of the cleaners' biocidal properties in the field. Bacterial and fungal growth varied among cemeteries.

Santa Fe National Cemetery displayed the largest amount of bacterial and fungal activity of the five cemeteries, which was five times greater than any other location. Jefferson Barracks results showed small quantities of fungal growth on all but one headstone. Fungi were found on headstones in both sunny and shady locations. Bacterial counts were limited to a few headstones in Jefferson Barracks. In Alexandria, more bacterial and fungal activity was seen on headstones in shady locations compared to sunny locations. Bacteria were not detected in many samples from San Francisco National Cemetery but, when



FIGURE 2. Biological growth observed on the test stone at Jefferson Barracks after six months: area A is treated with Kodak Photo-Flo, and area B is treated with H₂Orange, Grout Safe cleaner.

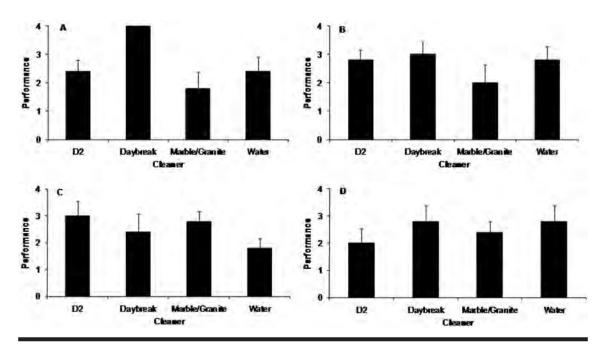


FIGURE 3. Bacterial and fungal growth on Colorado Yule marble headstones averaged for five cemeteries in two types of locations: (A) bacteria in sunny locations, (B) fungi in sunny locations, (C) bacteria in shaded locations, and (D) fungi in shaded locations. Error bars indicate the standard error of the mean.

found, were more likely to be seen in sunny locations. In contrast, bacteria and fungi were detected in few samples from Bath National Cemetery.

Initially, the presence of higher biological activity at Santa Fe National Cemetery seemed counterintuitive. Santa Fe is a drier climate, and little biological soiling had been observed in the cemetery. Locations such as Jefferson Barracks or Alexandria would be expected to have richer environments for biological growth because of their climates and higher relative humidities. It is important to note before evaluating results from initial biological analyses that each cemetery has its own regular maintenance schedule, which will influence the nature of the biological activity on headstones from that cemetery. For example, Santa Fe National Cemetery is the only one in the study where the stones have not been bleached as part of a regular maintenance schedule.

In terms of differences in location within the cemeteries, Daybreak showed better control of bacterial growth in sunny locations than the other treatments (Figure 3A); analysis of variance (ANOVA) confirmed a significant difference among the cleaners at a 95% confidence level, i.e., a probability level p < 0.05. No other significant differences were found, however, for fungi in sunny locations (Figure 3B), bacteria in shaded locations (Figure 3C), and fungi in shaded locations (Figure 3D).

Laboratory studies are planned to further evaluate the effectiveness of the three remaining cleaners, and accelerated studies will be carried out on stone samples in the Laboratory of Applied Microbiology at Harvard University. It must be noted, however, that none of the cleaners provide long-term protection against microbiological growth on stone.

ACKNOWLEDGMENTS

This work was funded by an interagency agreement between the U.S. Department of Veterans Affairs, the National Cemetery Administration (NCA), and the National Center for Preservation Technology and Training (NCPTT). More information about this study can be found in the project report at http://www.ncptt.nps.gov/interim-report-on-commercially-available-cleaners-for-use-on-federally-issued-headstones/.

REFERENCES

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Section 10

INTERAGENCY AGREEMENT BETWEEN DEPARTMENT OF VETERANS AFFAIRS AND NATIONAL PARK SERVICE

The agreement should contain the following provisions

I. PURPOSE

The Interagency Agreement (IAA) is between the Department of Veterans Affairs (VA), and National Park Service. The purpose of the agreement is to establish to terms and conditions under which the NPS shall provide a:

Materials Conservation and Treatment Analysis of marble, government-issued veteran headstones dating from the 1870s to about 1973 at five national cemeteries (TBD) in five geographically distinct regions. This study will serve as the scientific foundation for the development of a national policy on the appropriateness of headstone cleaning agents and their delivery within national cemeteries overseen by the National Cemetery Administration. It will provide relevant cleaning alternatives for managers of all cemeteries—run by a government or private—that contain veteran headstones, which are provided by the National Cemetery Administration.

This project involves a partnership composed of the NCA, which is the overall project administrator legally responsible for project work and funds, and the National Center for Preservation Technology and Training (NCPTT), an office of NPS.

II. <u>AUTHORITY</u>

This agreement is entered into pursuant to the authority of the Economy Act of 1932 (31 U.S.C. 1535) as amended, which authorizes the transfer of funds from one agency to another under an interagency agreement and where one Federal agency is authorize to provide services to another Federal agency. Additionally, legal authority exists for this acquisition, including a bona fide need, and is in the best interest of the Government in accordance with the Federal Acquisition Regulation (FAR 17.502).

NCPTT legislative authority to enter into the Agreement is P16 U.S.C. 470x-2, Sec. 403 which established within the Department of the Interior a National Center for Preservation Technology and Training whose purposes are to: develop and distribute preservation and conservation skills and technologies for the conservation of historic resources, take

steps to apply preservation technology benefits from on-going research by other agencies and institutions, and facilitate the transfer of preservation technology among Federal agencies, State and local governments, universities, international organizations and the private sector. NCPTT may enter into contracts and agreements with Federal, State, local and tribal governments to carry out its responsibilities under this title.

III. BACKGROUND

VA provides patient care and veterans' benefits—including burial-related entitlements—to 70 million veterans and eligible family members. An agency of VA, NCA maintains 3.6 million occupied gravesites in its 120 national cemeteries and 33 soldiers lots, which total more than 14,250 acres. NCA provides approximately 350,000 headstones/ markers annually to mark veteran graves in its cemeteries and those operated by other governments or privately.

NCPTT promotes and enhances the preservation and conservation of prehistoric and historic resources in the United States for present and future generations through the advancement and dissemination of preservation technology and training. It is an interdisciplinary program of the NPS to advance historic preservation technologies in the fields of archeology, historic architecture, and historic landscapes and objects and materials conservation. NCPTT serves public and private practitioners through research, education and information management.

IV. SCOPE OF WORK/STATEMENT OF WORK

NCPTT will:

- A. Develop a research project that studies the effects of commercially available cleaning solutions on government-issued marble headstones that were produced between the mid-1870s and 1975. Tests will include a range of commercially available products used to clean these headstones, to be evaluated using multiple criteria, including: cleaning effectiveness, inhibition of regrowth, ease of use and application, environmental and human safety, and potential long-term stone damage.
- B. Canvas cemetery stewards in private and VA national cemeteries to determine appropriate products/methods in current use for removal of biological growths on marble. NCPTT and NCA will jointly agree on a maximum of eight (8) products to be tested. Products will be applied according to manufacturer's recommendations.
- C. Choose products for testing that meet following criteria:

- User-friendly,
- Suitable for large-scale cleaning projects,
- Environmentally-friendly, and
- Cost-effective.
- D. <u>Phase I</u>: Test approximately a total of 1,440 headstones in typical national cemeteries in five (5) NCA regions, or Memorial Service Networks (MSNs): MSN II/Southeast, MSN I/Northeast, MSN V/Pacific West, MSN IV/Midwest, and MSN III/Intermountain. The testing will be executed in two phases. In the first phase, eight products will be tested in side-by-side test patches, approximately 4" x 4" in size, on headstones. Testing will be undertaken to accommodate the following variables per marble type at each site:
 - Various orientations (i.e., west face, east face)
 - Various environments (i.e., full sun, partial shade, full shade, and one other environmental condition TBD, such as water)
- E. Concurrent with the test patch studies in the field, a series of cut marble samples will be treated with each of the eight products and exposed beside the test patch stones. These samples will be used in both non-destructive and destructive laboratory testing. The purpose of the testing will be to detect residual cleaning products on the stone and to look at potential stone deterioration. Analytical methods will be selected that detect chemical and physical changes to the surface of the cut test stones.
- F. <u>Phase II</u>: Based on the results of test patches after at least nine months of study, up to four of the most effective products will be further tested on whole headstones. Whole stone studies will be monitored every three months for at least six months using the same techniques outlined for the test patch study.
- G. Submit its findings and recommendations in a final report to NCA. The report will include experimental and analytical results with conclusions and recommendations as to future studies that would include a broader array of stone.

NCA will:

- A. Provide financial support to NCPTT for the work described above.
- B. Assign staff to act as a liaison between NCPTT and NCA—for the project described herein.
- C. Assure the necessary access to the relevant cemeteries as needed.

- D. Assist in the selection of cemetery sites for the testing and identification of stones to be used as test markers.
- E. Supply headstones for laboratory test samples, preferably from the same field-test cemetery sites.
- F. Ensure that no headstone included in the field study is cleaned or otherwise treated (by staff or contractors) during the course of the research project.

Both parties agree to:

- A. Cooperate and coordinate to the fullest extent in the activities related to this Agreement so that the efforts of each party will produce the planned results.
- B. Consult frequently to discuss individual actions on the project and to assess progress according to plan.

<u>Acknowledgments of support and disclaimer:</u> An acknowledgment of NCA support must be made in connection with publications, audiovisuals, films, videos, journal articles, or public information of any kind based on, or developed under this project. This acknowledgment must be made in the form of a statement such as the following:

This document was developed with funds from the United States National Cemetery Administration. Its contents are solely the responsibility of the author and do not necessarily represent the official position or policies of the United States Department of Veterans Affairs, National Cemetery Administration, the National Park Service or the National Center for Preservation Technology and Training.

Publicity for the project (e.g., newspapers, radio and television releases, public talks, etc.,) should acknowledge the U.S.Department of Veterans Affairs, National Cemetery Administration. Consultants hired by NCPTT must be informed of this requirement.

V. FUNDING, 1358(a) or MIPR

Total funding under the terms of this agreement shall not exceed \$118,000. All reimbursement requests will be submitted in accordance with the Intragovernment Payment and Collection System (IPAC).

Appropriation: 3640129.001

ALC: 3600-00-1200 ACCode: 010070400 Cost Center: 512500 BOC: 2580

Obligation numbers TK

All expenses charged to the Agreement must be directly related to the approved scope of work and budget, and supported by approved contracts, purchase orders, requisitions, bills, or other evidence of liability consistent with generally established purchasing procedures and generally accepted accounting principles.

VI. POINTS OF CONTACT

David K. Schettler, Director Memorial Programs Service National Cemetery Administration Department of Veterans Affairs 810 Vermont Ave., NW (41A) Washington, DC 20420 202-501-3100 E-mail: david.schettler@mail.va.gov

Sara Amy Leach, Senior Historian National Cemetery Administration Department of Veterans Affairs 810 Vermont Ave., NW (41C4) Washington, DC 20420 202-565-6326 sara.leach@mail.va.gov

Henry J. Corback, Director Budget & Finance Service National Cemetery Administration Department of Veterans Affairs 810 Vermont Ave., NW (41B1) Washington, DC 20420 Telephone: 202-273-5157 henry.corback@mail.va.gov

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Cynthia Odonoo, Contracting Officer National Park Service 1201 Eye Street, NW, #2623 Washington, DC 20005 202-354-1946 202-565-1144 (fax)

VII. PERIOD OF AGREEMENT

This agreement shall become effective upon date of signature by both parties and shall remain in effect for a period of three years, unless extended by mutual consent of both parties. All products are due within 30 days after the end of project work stated in this Interagency Agreement.

VIII. <u>AMENDMENT AGREEMENT</u>

This agreement or any of its specific provisions may be revised or amended only by the signature approval of the party's signatory to the agreement or by their respective official successors.

IX. <u>TERMINATION OF AGREEMENT</u>

Either party upon 30 days notice in writing may accomplish termination of this agreement.

X. DISPUTE CLAUSE

Disputes concerning the interpretation of this agreement shall be resolved by a majority vote of a three-person dispute resolution committee. The committee shall consist of one VA representative, one NPS representative, and one neutral representative agreed upon by both VA and NPS.

XI. ACCEPTANCE BY BOTH PARTIES OF THE AGREEMENT

David S. Derr Deputy Assistant Secretary for Office of Acquisition and Material Management Department of Veterans Affairs	Date
Cynthia Odonoo Contracting Officer National Park Service Department of the Interior	Date

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This agreement is entered into pursuant to the authority of the Economy Act of 1932 (31 U.S.C. 1535) as amended, which authorizes the transfer of funds from one agency to another under an interagency agreement and where one Federal agency is authorize to provide services to another Federal agency. Additionally, legal authority exists for this acquisition, including a bona fide need, and is in the best interest of the Government in accordance with the Federal Acquisition Regulation (FAR 17.502).

NCPTT legislative authority to enter into the Agreement is P16 U.S.C. 470x-2, Sec. 403 which established within the Department of the Interior a National Center for Preservation Technology and Training whose purposes are to: develop and distribute preservation and conservation

skills and technologies for the conservation of historic resources, take steps to apply preservation technology benefits from on-going research by other agencies and institutions, and facilitate the transfer of preservation technology among Federal agencies, State and local governments, universities, international organizations and the private sector. NCPTT may enter into contracts and agreements with Federal, State, local and tribal governments to carry out its responsibilities under this title.

III. BACKGROUND

VA provides patient care and veterans' benefits—including burial-related entitlements—to 70 million veterans and eligible family members. An agency of VA, NCA maintains 3.6 million occupied gravesites in its 120 national cemeteries and 33 soldiers lots, which total more than 14,250 acres. NCA provides approximately 350,000 headstones/ markers annually to mark veteran graves in its cemeteries and those operated by other governments or privately.

NCPTT promotes and enhances the preservation and conservation of prehistoric and historic resources in the United States for present and future generations through the advancement and dissemination of preservation technology and training. It is an interdisciplinary program of the NPS to advance historic preservation technologies in the fields of archeology, historic architecture, and historic landscapes and objects and materials conservation. NCPTT serves public and private practitioners through research, education and information management.

IV. SCOPE OF WORK/STATEMENT OF WORK

NCPTT will:

A. Continue a research project that studies the effects of commercially available cleaning solutions on government-issued marble headstones that were produced between the mid-1870s and 1975. We will monitor the regrowth of bacteria, algae, and fungi on headstones previously cleaned with one of five commercially available cleaners. Monitoring will take place in Jefferson Barracks National Cemetery located in St. Louis, Mo. and Alexandria National Cemetery, located in Pineville, La.

• .

B. The two cemeteries will be evaluated annually for a period of two years. Evaluations will be based on color monitoring, changes in visual

appearance, and biological analyses as provided by the laboratory of applied microbiology, Harvard University.

C. Submit its findings and recommendations in a final report to NCA. The report will include experimental and analytical results with conclusions and recommendations as to future studies that would include a broader array of stone.

NCA will:

- A. Provide financial support to NCPTT for the work described above.
- B. Assign staff to act as a liaison between NCPTT and NCA—for the project described herein.
- C. Assure the necessary access to the relevant cemeteries as needed.
- D. Ensure that no headstone included in the field study is cleaned or otherwise treated (by staff or contractors) during the course of the research project.

Both parties agree to:

- A. Cooperate and coordinate to the fullest extent in the activities related to this Agreement so that the efforts of each party will produce the planned results.
- B. Consult frequently to discuss individual actions on the project and to assess progress according to plan.

<u>Acknowledgments of support and disclaimer:</u> An acknowledgment of NCA support must be made in connection with publications, audiovisuals, films, videos, journal articles, or public information of any kind based on, or developed under this project. This acknowledgment must be made in the form of a statement such as the following:

This document was developed with funds from the United States National Cemetery Administration. Its contents are solely the responsibility of the author and do not necessarily represent the official position or policies of the United States Department of Veterans Affairs, National Cemetery Administration, the National Park Service or the National Center for Preservation Technology and Training.

Publicity for the project (e.g., newspapers, radio and television releases, public talks, etc.,) should acknowledge the U.S.Department of Veterans Affairs, National Cemetery Administration. Consultants hired by NCPTT must be informed of this requirement.

V. FUNDING, 1358(a) or MIPR

Total funding under the terms of this agreement shall not exceed \$31,600. All reimbursement requests will be submitted in accordance with the Intragovernment Payment and Collection System (IPAC).

Appropriation: 3640129.001

ALC: 3600-00-1200 ACCode: 010070400 Cost Center: 512500

BOC: 2580

Obligation number: 101-J49242

All expenses charged to the Agreement must be directly related to the approved scope of work and budget, and supported by approved contracts, purchase orders, requisitions, bills, or other evidence of liability consistent with generally established purchasing procedures and generally accepted accounting principles.

VI. POINTS OF CONTACT

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NPS:

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Mary F. Striegel, NCPTT Materials Research Program Director National Center for Preservation Technology and Training 645 College Ave. Natchitoches, LA 71457 318-356-7444 318-356-9119 (fax) mary_striegel@nps.gov

Cynthia Odonoo, Contracting Officer National Park Service 1201 Eye Street, NW, #2623 Washington, DC 20005 202-354-1946 202-565-1144 (fax)

VII. PERIOD OF AGREEMENT

This agreement shall become effective upon date of signature by both parties and shall remain in effect for a period of three years, unless extended by mutual consent of both parties. All products are due within 30 days after the end of project work stated in this Interagency Agreement.

VIII. AMENDMENT AGREEMENT

This agreement or any of its specific provisions may be revised or amended only by the signature approval of the party's signatory to the agreement or by their respective official successors.

IX. <u>TERMINATION OF AGREEMENT</u>

Either party upon 30 days notice in writing may accomplish termination of this agreement.

X. <u>DISPUTE CLAUSE</u>

Disputes concerning the interpretation of this agreement shall be resolved by a majority vote of a three-person dispute resolution committee. The committee shall consist of one VA representative, one NPS representative, and one neutral representative agreed upon by both VA and NPS.

XI. ACCEPTANCE BY BOTH PARTIES OF THE AGREEMENT

David S. Derr Deputy Assistant Secretary for Office of Acquisition and Material Management Department of Veterans Affairs	Date
Cynthia Odonoo Contracting Officer National Park Service Department of the Interior	Date

INTERAGENCY AGREEMENT BETWEEN DEPARTMENT OF VETERANS AFFAIRS AND NATIONAL PARK SERVICE

Amendment 2

The agreement should contain the following provisions

I. PURPOSE

The Interagency Agreement (IA) is between the Department of Veterans Affairs (VA), and National Park Service. The purpose of the agreement is to establish the terms and conditions under which the NPS shall:

Continue materials conservation and treatment analysis of marble, government issued veteran headstones. This study will serve as the scientific foundation for the development of a national policy on the appropriateness of headstone cleaning agents and their delivery within national cemeteries overseen by the National Cemetery Administration. It will provide relevant cleaning alternatives for managers of all cemeteries—run by a government or private—that contain veteran headstones, which are provided by the National Cemetery Administration.

This project involves a partnership composed of the NCA, which is the overall project administrator legally responsible for project work and funds, and the National Center for Preservation Technology and Training (NCPTT), an office of NPS.

II. <u>AUTHORITY</u>

This agreement is entered into pursuant to the authority of the Economy Act of 1932 (31 U.S.C. 1535) as amended, which authorizes the transfer of funds from one agency to another under an interagency agreement and where one Federal agency is authorized to provide services to another Federal agency. Additionally, legal authority exists for this acquisition, including a bona fide need, and is in the best interest of the Government in accordance with the Federal Acquisition Regulation (FAR 17.502).

NCPTT legislative authority to enter into the Agreement is 16 U.S.C. 470x-2, Sec. 403 which established within the Department of the Interior a National Center for Preservation Technology and Training whose purposes are to: develop and distribute preservation and conservation skills and technologies for the conservation of historic resources, take steps to apply preservation technology benefits from on-going research by

other agencies and institutions, and facilitate the transfer of preservation technology among Federal agencies, State and local governments, universities, international organizations and the private sector. NCPTT may enter into contracts and agreements with Federal, State, local and tribal governments to carry out its responsibilities under this title.

III. BACKGROUND

In 2004 NCA and NCPTT entered into an interagency agreement to evaluate commercially available cleaners for use on federally-issued headstones. The goal of the research was to determine the effectiveness of the cleaners for removing biological growth and the length of time passing before re-growth was observed. Additional considerations included the ease of use of the treatment and the potential for long term stone damage. The work was carried out in the laboratory at NCPTT, through contractors at the Laboratory of Applied Microbiology, Harvard University, and in the field at five national cemeteries located in distinct geographic and climatic regions. Cemeteries included in this study were Alexandria National Cemetery in Pineville, LA; Bath National Cemetery in Bath, NY; Jefferson Barracks National Cemetery in St. Louis, MO; San Francisco National Cemetery, in San Francisco, CA; and Santa Fe National Cemetery, in Santa Fe, NM.

Water and five commercially available cleaners, including Sunshine Makers Inc. D/2Antimicrobial cleaner, Certified Labs' Daybreak cleaner, World Environmental Group Marble cleaner, H2Orange Grout Safe cleaner, and Kodak Photo-Flo were evaluated at each test cemetery. Cleaners were applied to test patches on headstones carved from Colorado Yule marble and White Cherokee Georgia marble. Testing also included sunny and shady locations to help account for possible differences arising from local environmental variations.

In field trials, changes to headstone test patches as a result of cleaning with test cleaners were evaluated by appearance change and biological activity. Laboratory studies looked for residual effects of cleaners including salt deposition that can lead to slow deterioration of the stone. Based on these results, two cleaners were eliminated from further consideration. Kodak Photo-Flo was a poor performer for the elimination of biological growth and did not inhibit re-growth on the headstones as evidenced in field studies. H₂Orange Grout Safe cleaner did not kill all microbes initially and left surface stains which vanished over time.

Field and laboratory studies continued on D/2 Antimicrobial cleaner, Daybreak cleaner, and World Monument Group Marble cleaner. Reoccurring biological activity was followed over eighteen month period in the field. Significant performance differences of these cleaners were not observed. Researchers associated with the project, including scientists at NCPTT and biologists at the Laboratory of Applied Microbiology, Harvard University, were concerned than an eighteen month time period may not have been sufficient to document significant visual changes or to allow for the growth of algae and photosynthetic bacteria. Laboratory tests indicated that D/2 Antimicrobial cleaner and Daybreak cleaner did leave soluble salts that could possibly affect long-term durability of the headstones.

Based on these results, the research project was extended through amendment 1 of the interagency agreement. The goal of additional research was to follow the reoccurrence of biological growth including bacteria, algae, and fungi on previously cleaned headstones in two cemeteries. Monitoring was to take place in Jefferson Barracks National Cemetery located in St. Louis, Mo. and in Santa Fe National Cemetery in Santa Fe, New Mexico. The two cemeteries were to be evaluated annually for a period of two years.

Due to a series of unforeseen events, headstones at Jefferson Barracks National Cemetery were cleaned without NCPTT's knowledge. Upon discovery by NCPTT staff at the first field evaluation, NCPTT and NCA consulted on alternatives to the field study. Amendment 2 to the interagency agreement redefines the scope of work needed to complete the purpose of the project, redirects all unused funds from Amendment 1, and provides additional funds to complete the study.

IV. SCOPE OF WORK/STATEMENT OF WORK

NCPTT will:

- A. Develop an experimental design in collaboration with a contractor to determine in a series of microbiological analyses which of three biocides is most effective in the protection of marble against re-growth of microbial films.
- B. Undertake research in collaboration with a contractor to analyze the effects of three biocides:
 - 1. Sunshine Makers Inc D/2 Biological Solution,
 - 2. Certified Laboratories Daybreak cleaner, and
 - 3. World Environmental Group's Marble and Granite cleaner.
- C. Prepare and provide marble samples and cleaners for the laboratory study. Analyses will be carried out on Cherokee white marble and Colorado Yule marble. Analyses will be carried out at each sampling time on five replicate samples of stone. Each sample will be analyzed for the presence of bacteria, fungi and algae. Analyses will be undertaken at monthly intervals in a laboratory environment.

D. Submit findings and recommendations in a final report to NCA. The report will detail the results of the laboratory study and summarize research efforts resulting from this interagency agreement.

NCA will:

- A. Provide financial support to NCPTT for the work described above.
- B. Assign staff to act as a liaison between NCPTT and NCA for the project described herein.

Both parties agree to:

- A. Cooperate and coordinate to the fullest extent in the activities related to this Agreement so that the efforts of each party will produce the planned results.
- B. Consult frequently to discuss individual actions on the project and to assess progress according to plan.
- C. <u>Acknowledgments of support and disclaimer:</u> An acknowledgment of NCA support must be made in connection with publications, audiovisuals, films, videos, journal articles, or public information of any kind based on, or developed under this project. This acknowledgment must be made in the form of a statement such as the following:

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Publicity for the project (e.g., newspapers, radio and television releases, public talks, etc.,) should acknowledge the U.S. Department of Veterans Affairs, National Cemetery Administration. Consultants hired by NCPTT must be informed of this requirement.

V. PLANNED PROJECT SCHEDULE

Month 1-2

1. Implement Amendment 2,

- 2. Prepare contracts,
- 3. Prepare stone samples
- 4. Acquire all supplies

Month 3-10

- 5. Contractor to begin laboratory analyses
- 6. Monitor biological growth on monthly basis for 6-8 months¹
- 7. Prepare written report on laboratory results for NCPTT Month 11-13
- 8. Review contractor results
- 9. Request any additional information
- Integrate lab results with field studies from previous phases of research
- 11. Draft final report

Month 14

- 12. Prepare revisions, corrections and clarifications to final report per requests by NCA
- 13. Submit final report

VI. FUNDING, 1358(a) or MIPR

Funding in the amount of \$34,335 was allocated to this project through Amendment 1 of IA V101(049A3)P-2004-036, dated July 27, 2008. To date funds were expended for field travel in the amount of \$1,782.23. This amendment redirects the remaining funds from Amendment 1 in the amount of \$32,552,77 for use to complete this project with the budget shown in Part VII.

Funds from Amendment 1 are as follows:

Appropriation: 3680129.001

ALC: 3600-00-1200 ACCode: 010070400 Cost Center: 512500

BOC: 2580

Obligation number 101-J89085

Additional funds under the terms of this amendment shall not exceed \$9,000 to complete this project (budget shown in Part VII). All reimbursement requests will be submitted in accordance with the Intra-government Payment and Collection System (IPAC).

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¹ Results will depend on biological growth rates. Experiments are expected to be completed in 6 months but may take longer.

ACCode: Cost Center:

BOC:

Obligation number:

All expenses charged to the Agreement must be directly related to the approved scope of work and budget supported by approved contracts, purchase orders, requisitions, bills or other evidence of liability consistent with the generally established purchasing procedures and generally accepted accounting principles.

VII. BUDGET

Item	Costs
Personnel	5,350
Contracted Biological	36,000
A I	

Analyses

Supplies 200

Total Costs 41,550

Funds remaining from 32,550

Amendment 1

Additional funding 9,000

VIII. POINTS OF CONTACT

NCA:

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Gina L White, Program Analyst Memorial Programs Service National Cemetery Administration 810 Vermont Ave., NW Washington, DC, 20420

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Email: gina.white@va.gov

Joan Jefferies, Director Budget & Finance Service National Cemetery Administration 810 Vermont Ave., NW (41B1) Washington, DC, 20420 Voice: (202) 461-6742

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NPS:

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Mary F. Striegel, NCPTT Materials Research Program Director National Center for Preservation Technology and Training 645 College Ave. Natchitoches, LA 71457 318-356-7444 318-356-9119 (fax) mary_striegel@nps.gov

Cynthia Adonoo, Contracting Officer National Park Service 1201 Eye Street, NW, #2623 Washington, DC 20005 202-354-1946 202-565-1144 (fax) Cynthia_adonoo@nps.gov

VII. PERIOD OF AGREEMENT

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VIII. AMENDMENT AGREEMENT

This agreement or any of its specific provisions may be revised or amended only by the signature approval of the party's signatory to the agreement or by their respective official successors.

IX. <u>TERMINATION OF AGREEMENT</u>

Either party upon 30 days notice in writing may accomplish termination of this agreement.

X. <u>DISPUTE CLAUSE</u>

Disputes concerning the interpretation of this agreement shall be resolved by a majority vote of a three-person dispute resolution committee. The committee shall consist of one VA representative, one NPS representative, and one neutral representative agreed upon by both VA and NPS.

XI. ACCEPTANCE BY BOTH PARTIES OF THE AGREEMENT

Anthony Carlisi	Date
Director, Business Services	
Center for Acquisition Innovation	
U.S. Department of Veterans Affairs	
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Consthing Adams of	
Cynthia Adonoo	Date
Contracting Officer	
National Park Service	
Department of the Interior	